adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* for male F344/N rats that received 100 mg/kg 2-amino-5-nitrophenol, as shown by the increased incidence of acinar cell adenomas of the pancreas. Reduced survival of male F344/N rats that received 200 mg/kg decreased the sensitivity of this group for detecting a carcinogenic response. There was *no evidence of carcinogenic activity* for female rats that received 100 or 200 mg/kg per day. Marginally increased incidences of preputial or clitoral gland adenomas or carcinomas (combined) occurred in male and female F344/N rats administered 200 mg/kg 2-amino-5-nitrophenol. There was *no evidence of carcinogenic activity* for $B6C3F_1$ mice that received 400 mg/kg 2-amino-5-nitrophenol; reduced survival of $B6C3F_1$ mice that received 800 mg/kg caused this group to be considered inadequate for detecting a carcinogenic response.

Report Date: February 1988

TR-335 Toxicology and Carcinogenesis Studies of C.I. Acid Orange 3 (CAS No. 6373-74-6) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

C.I. Acid Orange 3 is a dinitrodiphenylamine derivative used exclusively as a dye (up to 0.2%) in semipermanent hair coloring products. This study was one of a series on semipermanent hair dyes, which included HC Blue No. 1 (NTP TR 271), HC Blue No. 2 (NTP TR 293), HC Red No. 3 (NTP TR 281), and C.I. Disperse Blue 1 (NTP TR 299). Toxicology and carcinogenesis studies of C.I. Acid Orange 3 (90% pure, containing 10% water for short-term studies and containing 6%-8% water and 2%-4% acetone for 2-year studies) were conducted by administering the dye in corn oil by gavage to F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (at 94-1,500 mg/kg in rats and 62-1,000 mg/kg in mice), no compound-related deaths or body weight changes were observed and no adverse effects were observed at necropsy.

In the 13-week studies (at 94-1,500 mg/kg in rats and 31-2,000 mg/kg in mice), compound-related kidney lesions were observed in rats and mice of each sex. These lesions included variable degrees of degeneration and necrosis of epithelial cells in the proximal convoluted tubules, regeneration of tubular epithelium, and granular casts in the tubules. In a few female rats of the highest dose group, necrosis of the renal papillae and suppurative inflammation were also observed. Mean body weights were generally comparable among groups of rats and mice. Mice receiving 2,000 mg/kg had body weights 11%-12% lower than those of vehicle controls. Five of 10 female rats that received the highest dose of 1,500 mg/kg died before the end of the study, but no compound-related deaths occurred in male rats or mice of either sex.

Based on these results, 2-year studies of C.I. Acid Orange 3 were conducted by administering the dye by gavage in corn oil at 0, 375, or 750 mg/kg to groups of 50 F344/N rats of each sex, 5 days per week for 103 weeks. Groups of 50 male $B6C3F_1$ mice were administered 0, 125, or 250 mg/kg C.I. Acid Orange 3 on the same schedule, and groups of 50 female $B6C3F_1$ mice were administered 0, 250, or 500 mg/kg. These doses were selected on the basis of the nature and severity of the renal lesions in both species.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats were generally more than 10% lower than those of vehicle controls after week 52 for males and week 70 for females. Mean body weights for low dose groups were comparable to those of vehicle controls. The survival of high dose male (after week 33) and female (after week 14) rats was lower ($P<0.05$) than that of vehicle controls and was attributed to nephrotoxicity (final survival—male: vehicle control, 36/50; low dose, 30/50; high dose, 0/50; female: 43/50; 34/50; 7/50). Mean body weights of dosed male and female mice were lower than those of vehicle controls (high dose, 5%-11% after week 74; low dose, 7%-17% after week 48). Survival of both the low dose (after week 102) and high dose (after week 100) groups of male mice was lower than that of the vehicle controls (final survival: 38/50; 25/50; 26/50). Although survival was lower than usual, no notable differences in survival were observed between groups of female mice (final survival: 23/50; 23/50; 24/50).

Nonneoplastic and Neoplastic Lesions in the Two-Year Studies: For both species, the kidney was the major target organ for C.I. Acid Orange 3. These findings are summarized in the accompanying table. The incidences of renal pelvic epithelial hyperplasia were increased in dosed rats of each sex. No renal neoplasms were observed in dosed male rats, but a tubular cell adenocarcinoma was observed in a vehicle control male rat. Six transitional cell carcinomas of the kidney were observed in high dose female rats; kidney transitional cell neoplasms have not been observed in 1,697 corn oil vehicle control female F344/N rats.

Nonneoplastic lesions characteristic of secondary renal hyperparathyroidism or secondary to uremia also occurred in dosed rats. The lesions included parathyroid hyperplasia, fibrous dysplasia of bone, erosion and ulcers of the glandular stomach, and mineralization of the aorta and glandular stomach.

Epithelial hyperplasia of the urinary bladder was observed in one low dose and three high dose female mice. A squamous cell carcinoma was seen in the urinary bladder of one low dose female mouse. Even though no squamous cell urinary bladder neoplasms have been observed in 1,665 corn oil vehicle control female $B6C3F_1$ mice, this single neoplasm in a low dose animal was not considered to be related to the administration of C.I. Acid Orange 3.

Genetic Toxicology: C.I. Acid Orange 3 was mutagenic with and without exogenous metabolic activation in *Salmonella typhimurium* strains TA97; TA98; and TA100; no mutagenicity was observed for strain TA1535.

Audit: The data, documents, and pathology materials from the 2-year studies of C.I. Acid Orange 3 have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male F344/N rats administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered to be inadequate for assessment of carcinogenic activity. There was *clear evidence of carcinogenic activity* of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney. There was *no evidence of carcinogenic activity* of C.I. Acid Orange 3 for male B6C3F$_1$ mice administered 125 or 250 mg/kg or for female B6C3F$_1$ mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dose groups of both sexes of rats and mice.

Synonyms: 2-anilino-5-(2,4-dinitroanilino)-benzenesulfonic acid, monosodium salt; 5[(2,4-dinitrophenol)-amine]-2-(phenylamine)-benzenesulfonic acid, monosodium salt; C.I. 10385; Tetracid Light Yellow 2R

Report Date: December 1988

TR-336 Toxicology and Carcinogenesis Studies of Penicillin VK (CAS No. 132-98-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Penicillin VK, a widely used antibiotic for treatment of gram-positive coccal infections, was nominated for study by the National Cancer Institute because rodent carcinogenicity studies for this drug had not been performed. The chemical (94% or 98% pure, USP grade) was administered orally (by gavage in corn oil) because oral administration is the primary route used to treat infections in humans. Fourteen-day, 13-week, and 2-year studies were conducted in F344/N rats and B6C3F$_1$ mice. Additional studies were performed to evaluate the potential for genetic damage in bacteria and mammalian cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, penicillin VK was administered at doses of 150-2,400 mg/kg. No compound-related deaths or dose-related histopathologic lesions were seen in rats or mice. Final mean body weights of dosed male rats were 5%-17% lower than that of controls; weights of dosed and control female rats were comparable. Final mean body weights of dosed mice were 5%-9% lower than those of controls. Diarrhea was observed in all dosed groups of rats and mice.

In the 13-week studies, male and female rats received doses of 180-3,000 mg/kg and male and female mice received doses of 250-3,000 mg/kg. No compound-related deaths were seen in rats or mice. Final mean body weights of rats that received 3,000 mg/kg were 11% lower than those of the vehicle controls for males and 6% lower for females. For mice, mean body weights were comparable. Diarrhea occurred in male rats at doses of 750 mg/kg and above and in female rats at doses of 1,500 and 3,000 mg/kg. Mucous cell metaplasia of the glandular stomach was observed in male and female rats receiving 1,500 and 3,000 mg/kg. Lesions of the glandular stomach (inflammation, mucous cell metaplasia, and eosinophilic cytoplasmic change) and the forestomach (papillary hyperplasia and hyperkeratosis) were seen in all groups of dosed mice. The severity of lesions at 1,000 mg/kg or below was considered minimal. Based on these results, doses selected for rats and mice in the 2-year studies were 0, 500, or 1,000 mg/kg.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control male and female rats and male mice were comparable. Mean body weights of dosed female mice were 4%-16% lower than those of the vehicle controls from week 28 to the end of the study. Diarrhea was observed for dosed male and female rats and for dosed male mice. Survival of low and high dose male rats and high dose female rats was reduced (male rats: vehicle control, 34/50; low dose, 19/50; high dose, 16/50; female rats: 29/50; 26/50; 16/50). Survival of male and female mice was comparable to that of the vehicle controls (male mice: 24/50; 36/50; 26/50; female mice: 36/50; 32/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nonneoplastic lesions occurred at low incidences in the nasal mucosa, lung, and forestomach of dosed male rats and in the nasal mucosa and lung of dosed female rats. Congestion and aspiration pneumonia occurring in dosed rats dying before week 104 was the principal cause of death in these animals.

Nonneoplastic lesions of the gastric fundal gland (eosinophilic cytoplasmic change and dilatation) and glandular stomach (cyst, chronic focal inflammation, hyperplasia, fibrosis, and squamous metaplasia) were seen in dosed male and female mice, and lesions of the gallbladder (eosinophilic cytoplasmic change) were seen in male mice.

Slight increases in the incidences of adenomas of the pituitary gland in high dose male rats and of fibroadenomas or adenomas (combined) of the mammary gland in low dose female rats were observed. These were not considered to be compound-related lesions.

The incidence of hepatocellular adenomas was decreased in high dose male mice (14/50; 15/49; 4/49). No compound-related neoplasms were seen in female mice.

Genetic Toxicology: Penicillin VK was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. The chemical was mutagenic only with activation in the mouse lymphoma L5178Y/TK$^{+/-}$ forward mutation

assay. Incubation of Chinese hamster ovary cells with penicillin VK resulted in increased frequencies of sister chromatid exchanges and chromosomal aberrations in the absence of metabolic activation under the conditions of delayed harvest to compensate for chemical-induced cell cycle delay, no effects from penicillin VK exposure were observed in these cells in the presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of penicillin VK were audited. The audit findings show that the conduct of the studies is documented and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of penicillin VK for F344/N rats or for B6C3F$_1$ mice administered 500 or 1,000 mg/kg penicillin VK in corn oil gavage, 5 days per week for 2 years. Nonneoplastic lesions were seen in the glandular stomach of dosed mice. Decreased survival of low and high dose male rats and of high dose female rats reduced the sensitivity of the studies for determining the presence or absence of a carcinogenic response in this species.

Synonyms: 4-thia-1-azabicyclo(3.2.0)heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-(2-phenoxyacetamide)-, monopotassium salt; penicillin V potassium; penicillin V potassium salt; D-α-phenoxymethylpenicillinate K salt; phenoxymethylpenicillin potassium; PVK

Trade Names: Antibiocin; Apsin VK; Aracil; Arcasin; Aspin VK; Beromycin; Beromycin 400; Betapen VK; Calciopen K; Cliacil; Compocillin VK; Distakaps V-K; Distaquaine V-K; Dowpen V-K; DQV-K; Fenoxypen; Icipen; Isocillin; Ispenoral; Ledercillin VK; Megacillin oral; Oracil-VK; Orapen; Ospeneff; Pedipen; Penagen; Pencompren; Pen-Vee K; Pen-V-K powder; Penvikal; Pfizerpen VK; Qidpen VK; Robicillin VK; Rocillin-VK; Roscopenin; SK-Penicillin VK; Stabilin VK Syrup 125; Stabilin VK Syrup 62.5; Sumapen VK; Suspen; Uticillin VK; V-Cil-K; V-Cillin K; Veetids; Vepen

Report Date: June 1988

TR-337 Toxicology and Carcinogenesis Studies of Nitrofurazone (CAS No. 59-87-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Nitrofurazone is a synthetic furan derivative, active against a broad spectrum of bacteria, which has been widely used in veterinary and human medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing nitrofurazone (99% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: Groups of five males and five females of each species were fed diets containing 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm for 14 consecutive days. Early deaths occurred in all groups of rats receiving 5,000 or 10,000 ppm nitrofurazone. The surviving rats in the lower two dose groups gained weight, but weight gain was decreased as the dose of nitrofurazone was increased. Feed consumption by rats of each sex was decreased at all doses above 630 ppm. In all dosed groups, clinical signs of toxicity included rough hair coats and lethargy. At doses of 2,500 ppm and above, rats of each sex exhibited intermittent episodes of seizures and lethargy.

All mice that received 2,500, 5,000, or 10,000 ppm nitrofurazone and 3/5 males that received 1,250 ppm died before the end of the 14-day studies; the surviving dosed mice (except females at 630 ppm) lost weight. A dose-related decrease in feed consumption was observed at all doses above 630 ppm. Clinical signs included rough hair coats and convulsive seizures.

In the 13-week studies, groups of 10 rats of each sex were given diets containing 0, 150, 310, 620, 1,250, or 2,500 ppm nitrofurazone. No deaths were observed and all animals gained weight, but the magnitude of weight gain was dose dependent with decrements in final mean body weight for the highest dose group reaching 55% in males and 36% in females. Other evidence of chemically related toxicity included convulsive seizures, osteoporosis, degenerative arthropathy, and gonadal hypoplasia in both sexes at the two highest doses.

Groups of 10 mice of each sex were given diets containing 0, 70, 150, 310, 620, or 1,250 ppm nitrofurazone for 13 weeks. Early deaths were observed in the two highest dose groups of each sex. The final mean body weights of male and female mice in the 1,250-ppm groups were about 20% lower than those of the controls; weight gains of the other dosed mice were comparable to those of the controls. Stimulus-induced convulsive seizures were observed for all mice in the two highest dose groups. Testicular hypoplasia was observed in the two highest dose groups of male mice.

Body Weight and Survival in the Two-Year Studies: Dietary concentrations for the 2-year studies were 0, 310, or 620 ppm for rats and 0, 150, or 310 ppm for mice (50 animals per dose group). Mean body weights of high dose male rats were lower than those of the controls after week 39; mean body weights of low dose male rats and of the controls were comparable throughout the study. Final mean body weights of low and high dose female rats were 9% and 21% lower than those of the controls. Dosed rats consumed less feed than did the controls. The average amount of nitrofurazone consumed per day was approximately 11-12 or 24-26 mg/kg by low or high dose male and female rats. The survival of the high dose group of male rats was lower than that of the controls after week 92 (final survival—male: control, 33/50; low dose, 30/50; high dose, 20/50; female: 28/50; 37/50; 31/50).

Mean body weights of dosed mice were similar to or somewhat greater than those of controls throughout most of the studies. The average daily feed consumption by dosed mice was similar to that of controls. The average amount of nitrofurazone consumed per day was approximately 14-16 or 29-33 mg/kg for low or high dose male and female mice. The survival of the high dose group of male mice was lower than that of the controls after week

88 (final survival—male: 39/50; 31/50; 27/50; female: 39/50; 40/50; 35/50).

In mice of each sex, nitrofurazone administration induced stimulus-sensitive convulsive seizures beginning at week 4 or 5 for high dose mice and week 24 for low dose female mice. These seizures were observed primarily in the first year of the study.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Degenerative changes involving the vertebral and femoro-tibial (knee) joints were observed at increased incidences in dosed rats. The degenerative changes primarily affected the articular cartilage and were similar to those seen in the 13-week studies. Degeneration of the sternal synchondroses was increased in high dose female rats. The osteoporosis seen in the 13-week studies was not observed in the 2-year studies. Testicular degeneration, characterized by atrophy of the germinal epithelium and aspermatogenesis, was observed at increased incidences in dosed male rats (control, 12/50; low dose, 49/50; high dose, 47/50).

Adenomas of the sebaceous glands and trichoepitheliomas or sebaceous adenomas (combined) of the skin were observed in high dose male rats (0/50; 0/50; 5/50). Carcinomas of the preputial gland were increased in dosed male rats (1/50; 8/50; 5/50). The incidences of preputial gland adenomas or carcinomas (combined) in dosed male rats were not statistically greater than that in the controls (9/50; 16/50; 7/50). However, in the low dose group, the incidence is greater than the highest incidence observed in historical untreated control groups (9/50). In addition, hyperplasia of the preputial gland was observed in six low dose male rats in which neither adenomas nor carcinomas occurred. The incidence of mesotheliomas of the tunica vaginalis in low dose male rats was greater than that in the controls (0/50; 7/50; 2/50).

Fibroadenomas of the mammary gland occurred at markedly increased incidences in dosed female rats (8/49; 36/50; 36/50). Three adenocarcinomas were also observed (1/49; 0/50; 2/50).

Ovarian atrophy (7/47; 44/50; 38/50) and tubular cell hyperplasia of the ovary (1/47; 23/50; 21/50) were observed at markedly increased incidences in dosed female mice. The incidences of benign mixed tumors (0/47; 17/50; 20/50), granulosa cell tumors (1/47; 4/50; 9/50), and granulosa cell tumors or luteomas (combined) (3/47; 6/50; 9/50) of the ovary were increased in exposed female mice.

Mononuclear cell leukemia in rats occurred with negative trends (male: 21/50; 23/50; 6/50; female: 15/49; 2/50; 2/50). In female mice, the incidences of adenomas or carcinomas (combined) of the anterior pituitary gland occurred with a negative trend (10/50; 7/50; 2/49). The incidences of testicular interstitial cell tumors were decreased in dosed male rats (45/50; 30/50; 28/50).

Genetic Toxicity: Nitrofurazone was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 both with and without exogenous metabolic activation. The responses in strains TA1535 and TA1537 were more varied: nitrofurazone was mutagenic in strain TA1535 only in the presence of S9 and produced no consistent increase in gene reversions in strain TA1537 with or without S9. In the absence of metabolic activation, nitrofurazone induced forward mutations at the $TK^{+/-}$ locus of mouse L5178Y lymphoma cells; the chemical was not tested with S9. Treatment of cultured Chinese hamster ovary cells with nitrofurazone in the absence of S9 produced a dose-related increase in sister chromatid exchanges and chromosomal aberrations; with S9, sister chromatid exchanges were increased, but no induction of chromosomal aberrations was observed.

Audit: The data, documents, and pathology materials from the 2-year studies of nitrofurazone were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of nitrofurazone for male F344/N rats as shown by the occurrence of sebaceous gland adenomas and trichoepitheliomas of the skin, mesotheliomas of the tunica vaginalis, and preputial gland tumors. There was *clear evidence of carcinogenic activity* of nitrofurazone for female F344/N rats as shown by a markedly increased incidence of fibroadenomas of the mammary gland. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing nitrofurazone at concentrations of 150 or 310 ppm. There was *clear evidence of carcinogenic activity* of nitrofurazone for female $B6C3F_1$ mice as shown by increased incidences of benign mixed tumors and granulosa cell tumors of the ovary.

Administration of nitrofurazone was associated with decreased incidences of mononuclear cell leukemia in male and female rats, testicular interstitial cell tumors in male rats, and pituitary gland neoplasms in female mice. Convulsive seizures in mice of each sex, ovarian atrophy in female mice, testicular degeneration in rats, and degeneration of articular cartilage in rats were all associated with the administration of nitrofurazone.

Synonyms: 5-nitro-2-furaldehyde semicarbazone; 2-[(5-nitro-2-furanyl)methylene]hydrazine carboximide

Trade Names: Aldomycin; Amifur; Chemfuran; Coxistat; Furacin; Furacinetten; Furaplast; Furazol W; Furesol; Furracoccid; Mammex; Nefco; Nifuzon; Nitrofural; Vabrocid

Report Date: June 1988

TR-338 Toxicology and Carcinogenesis Studies of Erythromycin Stearate (CAS No. 643-22-1) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies) TR-338

Toxicology and carcinogenesis studies of erythromycin stearate (USP grade, greater than 96% pure) were conducted by administering the antibiotic in feed to groups

of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Erythromycin stearate was studied because of its widespread use in humans as a broad-spectrum macrolide antibiotic and because of the lack of adequate long-term studies for carcinogenicity.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, none of the rats (at dietary concentrations up to 50,000 ppm) and 2/5 female mice that received 50,000 ppm died before the end of the studies. Final mean body weights of male rats that received 12,500, 25,000, or 50,000 ppm were 10%, 30%, or 36% lower, respectively, than that of controls; final mean body weights of female rats were 10%, 12%, or 32% lower. None of the dosed mouse groups gained weight. The final mean body weight of male mice that received 50,000 ppm was 10% lower than that of controls.

In the 13-week studies, none of the rats or mice (at dietary concentrations up to 20,000 ppm) died before the end of the studies. Final mean body weights of the 20,000-ppm groups of rats were more than 12% lower than that of the controls for males and 7% lower for females. Final mean body weights of mice that received 10,000 or 20,000 ppm were 15% or 19% lower than that of controls for males and 5% or 14% lower for females.

Multinucleated syncytial hepatocytes were observed in 10/10 male rats that received 20,000 ppm but in 0/10 male rats that received 10,000 ppm. No compound-related gross or microscopic pathologic effects were observed in mice.

Based on these results, 2-year studies of erythromycin stearate were conducted by feeding diets containing 0, 5,000, or 10,000 ppm erythromycin stearate to groups of 50 rats of each sex for 103 weeks. Diets containing 0, 2,500, or 5,000 ppm were fed to groups of 50 mice of each sex for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were comparable to those of controls throughout the studies. Mean body weights of high dose female rats were 5%-10% lower than those of controls. Mean body weights of dosed and control mice were comparable. The average daily feed consumption was similar for dosed and control male and female rats. For mice, estimated daily feed consumption by low and high dose males was similar to that of the controls and by low and high dose females was 92% that of the controls. The average amount of erythromycin stearate consumed per day was approximately 180 or 370 mg/kg for male rats and 210 or 435 mg/kg for female rats; for mice, the average amounts were 270 or 545 mg/kg for males and 250 or 500 mg/kg for females.

No significant differences in survival were observed between any groups of rats or mice of either sex (final survival – male rats: control, 28/50; low dose, 23/50; high dose, 27/50; female rats: 29/50; 30/50; 38/50; male mice: 34/50; 33/50; 40/50; female mice: 38/50; 34/50; 40/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Granulomas of the liver were observed at increased incidences in high dose rats (male: 1/50; 2/50; 10/50; female: 18/50; 27/50; 43/50). Granulomatous inflammation or granulomas of the spleen were observed in dosed female rats (0/48; 1/49; 3/50). Reticulum cell hyperplasia in the bone marrow occurred at increased incidences in high dose female rats (10/50; 14/50; 25/50).

Squamous cell papillomas of the oral mucosa were observed in 1/50 control, 2/50 low dose, and 3/50 high dose female rats. These tumors were considered to be marginal and not related to exposure. Hyperplasia of the oral mucosa was not observed.

Pheochromocytomas of the adrenal gland in female rats occurred with a positive trend (1/50; 4/49; 5/50). The incidences in the dosed groups are similar to the average historical incidence (9%) of this tumor in untreated control female F344/N rats at the study laboratory. This marginal tumor increase is not considered to be biologically important. No increases in incidences of neoplasms were observed at any site in dosed male rats.

Inflammation in the glandular stomach was observed at increased incidences in dosed male mice (1/49; 4/50; 6/50). Lymphoid hyperplasia in the urinary bladder was observed at increased incidences in dosed female mice (1/50; 9/47; 7/48).

No increases in incidences of neoplasms were observed at any site in dosed male or female mice.

Genetic Toxicology: Erythromycin stearate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested both with or without exogenous metabolic activation. Erythromycin stearate demonstrated equivocal mutagenicity in the mouse L5178Y lymphoma cell assay in the absence of exogenous metabolic activation (S9); erythromycin stearate was not mutagenic in the presence of S9. Treatment of cultured Chinese hamster ovary cells with erythromycin stearate did not produce an increase in the frequency of sister chromatid exchanges or chromosomal aberrations in either the presence or absence of metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of erythromycin stearate have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of erythromycin stearate for male or female F344/N rats administered erythromycin stearate in the diet at 5,000 or 10,000 ppm. There was *no evidence of carcinogenic activity* of erythromycin stearate for male or female B6C3F$_1$ mice administered erythromycin stearate in the diet at 2,500 or 5,000 ppm. Dose-related increases in the incidences of granulomas of the liver were observed in male and female rats. The absence of any biologically important chemical-associated effects in mice suggests that higher doses could have been given to male and female mice.

Synonyms: erythrocin stearate; erythromycin octadecanoate

Trade Names: Abbotcine; Bristamycin; Dowmycin E; Eratrex; Erypar; Ethril; Gallimycin; HSDB 4178; OE 7; Pantomicina; Pfizer-E; SK-Erythromycin; Wyamycin S

Report Date: December 1988

TR-339 Toxicology and Carcinogenesis Studies of 2-Amino-4-Nitrophenol (CAS No. 99-57-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

2-Amino-4-nitrophenol is used to color semipermanent hair dyes and in the manufacture of mordant dyes for leather, nylon, silk, wool, and fur. 2-Amino-4-nitrophenol was nominated by the National Cancer Institute for toxicology and carcinogenesis studies because of widespread human exposure associated with its manufacture and use. Toxicology and carcinogenesis studies were conducted by administering 2-amino-4-nitrophenol (98% pure) in corn oil by gavage, 5 days per week, to groups of F344/N rats and B6C3F$_1$ mice of each sex in 15-day, 13-week, and 2-year studies.

Fifteen-Day and Thirteen-Week Studies: During the 15-day studies, rats and mice received doses of 0, 313, 625, 1,250, 2,500, or 5,000 mg/kg. All rats that received 2,500 or 5,000 mg/kg and all female rats that received 1,250 mg/kg died before the end of the studies. Final mean body weights of chemically exposed rats surviving to the end of the studies were comparable to those of vehicle controls. Diarrhea was observed in all groups of exposed rats except those receiving 313 mg/kg. All mice that received 2,500 or 5,000 mg/kg, 2/5 males and all females that received 1,250 mg/kg, and 1/5 females that received 313 mg/kg died before the end of the studies. Final mean body weights of exposed mice surviving until the end of the studies were comparable to those of vehicle controls.

In 13-week studies, F344/N rats and B6C3F$_1$ mice of each sex received 2-amino-4-nitrophenol at doses of 0, 62.5, 125, 250, 500, or 1,000 mg/kg. All rats that received 1,000 mg/kg and 2/10 males and 2/10 females that received 500 mg/kg died before the end of the studies. The final mean body weight of male rats that received 500 mg/kg was reduced 10% compared with that of vehicle controls; final mean body weights of all other surviving exposed rat groups were comparable to those of vehicle controls. Diarrhea and lethargy were observed for rats that received 500 or 1,000 mg/kg. All male mice and most females that received 1,000 mg/kg and 4/10 females that received 500 mg/kg died before the end of the studies. Final mean body weights of chemically exposed mice were comparable to those of vehicle controls. No compound-related clinical signs were observed in mice during the studies.

Mineralization of the renal cortex and degeneration of the renal tubular epithelium were observed in male and female rats that received 1,000 mg/kg and in males that received 500 mg/kg. Degeneration and necrosis of the renal tubular epithelium was observed in 5/10 male and 3/10 female mice that received 1,000 mg/kg.

Body Weight and Survival in the Two-Year Studies: In the 2-year studies, rats and mice received 2-amino-4-nitrophenol at doses of 0, 125, or 250 mg/kg. Mean body weights of male rats that received 250 mg/kg were 8%-10% lower than those of vehicle controls throughout most of the 2-year study. Mean body weights of female rats were comparable to those of vehicle controls. Soft stools and occasional diarrhea were observed in chemically exposed rats starting 6 months after the beginning of the studies. Survival of male rats that received 250 mg/kg was markedly lower than that of vehicle controls after week 89 (final survival: vehicle control, 32/50; 125 mg/kg group, 24/50; 250 mg/kg group, 10/50). Survival of female rats was comparable among all groups (final survival: 25/50; 27/50; 31/50).

Mean body weights of male and female mice that received 250 mg/kg were comparable to those of vehicle controls; the mean body weights of female mice that received 125 mg/kg were as much as 17% greater than that of vehicle controls. Survival of all mouse groups was comparable during the 2-year studies (final survival: male—28/50; 29/50; 23/50; female—28/50; 31/50; 30/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Pigmentation of the small and large intestines was present in exposed rats but not in vehicle controls. Ulcers and erosive lesions of the digestive tract were observed in male rats that received 250 mg/kg and to a lesser extent in male rats that received 125 mg/kg. A carcinoma of the colon occurred in one male rat that received 250 mg/kg; no other neoplasms were observed in the gastrointestinal tract of rats. No pigmentation, ulcers, or erosive lesions were found in the digestive tract of mice.

The severity of nephropathy was markedly greater in exposed male rats than in vehicle controls. Associated with the nephropathy were nonneoplastic lesions indicative of reduced renal function and secondary hyperparathyroidism, including parathyroid hyperplasia, mineralization of various organs, and fibrous osteodystrophy.

Renal tubular cell hyperplasia (1/50; 4/48; 5/50) and renal cortical (tubular cell) adenomas (0/50; 1/48; 3/50) occurred in male rats. Renal cortical adenomas are infrequently observed in male F344/N rats (historical incidence, 0.5%).

More preputial gland adenomas or carcinomas (combined) were observed in low dose male rats than in vehicle controls (3/50; 10/48; 3/50), whereas the incidences of clitoral gland neoplasms were decreased in dosed female rats (9/50; 6/50; 1/49).

Hemangiomas or hemangiosarcomas (combined) occurred in male mice that received 2-amino-4-nitrophenol (0/50; 1/50; 5/50); each tumor was present at a different site. The historical control incidence is 11% at the study laboratory and 6% in 2-year NTP studies.

Genetic Toxicology: 2-Amino-4-nitrophenol was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 with metabolic activation. 2-Amino-4-nitrophenol was not mutagenic in strains TA1535 or TA1537. 2-Amino-4-nitrophenol was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay without metabolic activation. It was not tested with activation. 2-Amino-4-

nitrophenol induced sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary cells in the presence and absence of metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of 2-amino-4-nitrophenol were audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of 2-amino-4-nitrophenol for male F344/N rats, as shown by increased incidences of renal cortical (tubular cell) adenomas. The incidences of renal tubular cell hyperplasia were also increased in male rats exposed to 2-amino-4-nitrophenol. The survival of male rats that received 2-amino-4-nitrophenol was reduced compared with survival of vehicle control male rats. There was *no evidence of carcinogenic activity* of 2-amino-4-nitrophenol for female F344/N rats or for male or female B6C3F$_1$ mice that received 125 or 250 mg/kg per day.

Report Date: June 1988

TR-340 Toxicology and Carcinogenesis Studies of Iodinated Glycerol (Organidin®) (CAS No. 5634-39-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of iodinated glycerol (Organidin®, a complex mixture prepared by the reaction of iodine with glycerol and found to contain 33% 3-iodo-1,2-propanediol as the major component) were conducted because of human exposure to iodinated glycerol as an expectorant and its possible relationship to the formation of alkyl iodides, e.g., methyl iodide, a suspected animal carcinogen. These studies were conducted by giving iodinated glycerol in water by gavage (5 days per week) to groups of F344/N rats and B6C3F$_1$ mice for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted with iodinated glycerol in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, Chinese hamster ovary (CHO) cells, and B6C3F$_1$ mice (in vivo bone marrow micronucleus test). Also, 3-iodo-1,2-propanediol was tested in *S. typhimurium* and B6C3F$_1$ mice (in vivo micronucleus assay).

Sixteen-Day and Thirteen-Week Studies: Sixteen-day studies were conducted by giving iodinated glycerol at doses up to 1,000 mg/kg to rats and up to 500 mg/kg to mice. All female rats and 4/5 male mice in the highest dose group died before the end of the studies; there were no dose-related effects on body weights of male or female rats or male mice at the end of the studies. The forestomach of 2/5 female mice that received 500 mg/kg was thickened and granular.

Thirteen-week studies were conducted by administering iodinated glycerol at doses up to 500 mg/kg to rats and mice. During these studies, 3/10 female rats and 1/10 female mice that received 500 mg/kg died. Final mean body weights of rats and mice that received 500 mg/kg were 4% lower than those of vehicle controls for males and 6%-7% lower for females.

Kidney tubular cell lesions, including cortical necrosis, regeneration, and calcification, were observed at increased incidences in the highest dose group of female rats. Lymphoid hyperplasia of the stomach was observed in dosed male and female rats. Kidney tubular cell regeneration was also observed in dosed female mice. Inflammation or abscesses of mild-to-moderate severity and hyperplasia, acanthosis, and/or hyperkeratosis of mild-to-moderate severity were observed in the forestomach of the highest dosed group of female mice.

Body Weight and Survival in the Two-Year Studies: Two-year studies were conducted by administering 0, 125, or 250 mg/kg iodinated glycerol in deionized water by gavage, 5 days per week for 103 weeks, to groups of 50 male F344/N rats and 50 male B6C3F$_1$ mice. Groups of 50 female F344/N rats and 50 female B6C3F$_1$ mice were administered iodinated glycerol on the same schedule at lower doses of 0, 62, or 125 mg/kg because of the increased severity of kidney and stomach lesions in the 13-week studies. Mean body weights of high dose male rats were 5%-10% lower than those of vehicle controls from week 43 to week 68 and 10%-13% lower from week 72 to the end of the studies. Mean body weights of low dose male rats and high dose female rats were 4%-9% lower than those of vehicle controls from week 88 to the end of the studies. The survival of the high dose group of male rats was considerably lower than that of the vehicle controls after week 86. No other significant differences in survival were observed between any groups of rats of either sex (male: vehicle control, 28/50; low dose, 20/50, high dose, 2/50; female: 31/50; 30/50; 27/50). Mean body weights of dosed and vehicle control male mice were similar. Mean body weights of high dose female mice were 6%-8% lower than those of vehicle controls from week 40 to week 64 and were 9%-13% lower thereafter. No significant differences in survival were observed between any groups of mice of either sex (male: 36/50; 40/50; 32/50; female: 40/50; 33/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidence of mononuclear cell leukemia were increased in dosed male rats (vehicle control, 14/50; low dose, 29/50; high dose, 24/50).

Follicular cell carcinomas of the thyroid gland in male rats occurred at an increased incidence in low dose male rats (0/49; 5/49; 1/49). Reduced survival of high dose male rats may have been responsible for the decreased tumor incidence in this group relative to that in the low dose group. Follicular cell carcinomas were observed in one low dose and one high dose female rat. Follicular cell carcinomas of the thyroid gland have been observed in 3/293 water gavage vehicle control male F344/N rats and in 10/1,904 untreated control male F344/N rats.

Adenomas of the nasal cavity were observed in two high dose male rats. Adenomas of the nasal cavity have

not been observed in 300 water gavage vehicle control male F344/N rats or in 1,936 untreated control male F344/N rats.

Squamous metaplasia and focal atrophy of the salivary glands were observed at increased incidences in dosed rats (squamous metaplasia—male: 0/48; 47/50; 48/49; female: 1/49; 48/50; 49/50; focal atrophy—male: 1/48; 10/50; 30/49; female: 0/49; 4/50; 11/50).

In dosed female mice, adenomas of the anterior pituitary gland were increased (10/47; 15/45; 24/46). The incidences of adenomas of the harderian gland in dosed female mice were increased (6/50; 8/40; 13/50). A carcinoma of the harderian gland was observed in another high dose female mouse.

Dilatation of the thyroid gland follicle and follicular cell hyperplasia were observed at increased incidences in dosed mice (dilatation—male: 0/48; 28/50; 32/50; female: 4/48; 11/48; 10/48; hyperplasia—male: 3/48; 46/50; 34/50; female: 2/48; 25/48; 35/48). The incidences of follicular cell adenomas were 3/48, 6/50, and 0/50 for males and 2/48, 3/48, and 4/48 for females.

Hyperkeratosis and acanthosis of the forestomach were observed at increased incidences in high dose male mice (hyperkeratosis: 0/49; 0/49; 5/50; acanthosis: 0/49; 1/49; 5/50). Squamous cell papillomas were observed in female mice (1/49; 2/50; 5/49). The historical incidence of forestomach squamous cell neoplasms is 4/339 (1.2%) in water gavage vehicle control female $B6C3F_1$ mice and is 18/1,994 (0.9%) in untreated control female $B6C3F_1$ mice. Squamous cell neoplasms were not observed in male mice.

Genetic Toxicology: Treatment of the base-substitution mutant *S. typhimurium* strains TA100 and TA1535 with iodinated glycerol in a preincubational protocol with and without S9 resulted in a dose-related increase in the number of revertant colonies; no increase in revertants was observed with the frame-shift mutant strains TA98 or TA1537. 3-Iodo-1,2-propanediol was also mutagenic in TA100 with or without S9; it was not mutagenic in TA98. Iodinated glycerol increased the number of trifluorothymidine-resistant cells in mouse lymphoma L5178Y/$TK^{+/-}$ assay in the absence of exogenous metabolic activation; it was not tested with activation. Iodinated glycerol induced sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells without S9; with S9, the frequency of SCEs was increased more than without S9 but no chromosomal aberrations were induced. No increase in micronucleated polychromatic erythrocytes was observed in the bone marrow of $B6C3F_1$ mice after injection with either iodinated glycerol or 3-iodo-1,2-propanediol.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* for male F344/N rats administered iodinated glycerol, as indicated by increased incidences of mononuclear cell leukemia and follicular cell carcinomas of the thyroid gland. Adenomas of the nasal cavity in two high dose male rats may have been related to the administration of iodinated glycerol. There was *no evidence of carcinogenic activity* for female F344/N rats administered 62 or 125 mg/kg iodinated glycerol by gavage for 103 weeks. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice administered 125 or 250 mg/kg iodinated glycerol by gavage for 103 weeks. There was *some evidence of carcinogenic activity* for female $B6C3F_1$ mice administered iodinated glycerol, as indicated by increased incidences of adenomas of the anterior pituitary gland and neoplasms of the harderian gland. Squamous cell papillomas of the forestomach may have been related to the administration of iodinated glycerol.

Significant nonneoplastic lesions considered related to exposure of iodinated glycerol were squamous metaplasia and focal atrophy of the salivary gland in male and female rats. Dilatation of the thyroid gland follicle and follicular cell hyperplasia were observed in male and female mice.

Synonyms or Trade Names: Organidin®; iodopropylidene glycerol

Report Date: March 1990

TR-341 Toxicology and Carcinogenesis Studies of Nitrofurantoin (CAS No. 67-20-9) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Nitrofurantoin was studied and evaluated because of its widespread use as a drug for treating urinary tract infections in humans, its structural relationship to known carcinogenic 5-nitrofuran compounds, and the lack of adequate studies to assess its carcinogenicity. Toxicology and carcinogenesis studies of nitrofurantoin were conducted by administering nitrofurantoin (greater than 99% pure) in feed to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: None of the rats (at dietary concentrations up to 20,000 ppm) died before the end of the 14-day studies. Rats that received 5,000, 10,000, or 20,000 ppm lost weight. Four of five male and 4/5 female mice that received 10,000 ppm and 1/5 females that received 5,000 ppm nitrofurantoin died before the end of the studies. Mice that received 5,000 ppm and male mice that received 10,000 ppm lost weight.

In the 13-week studies, final mean body weights of rats that received 2,500, 5,000, or 10,000 ppm were 10%, 34%, or 47% lower than that of the controls for males and 15%, 31%, or 41% lower for females. Feed consumption by dosed and control rats was generally similar. Degeneration of the germinal epithelium of the seminiferous tubules of the testis was observed in male rats that received 2,500 to 10,000 ppm nitrofurantoin. Necrosis of the ovarian follicles was observed in 8/10 female rats that received 10,000 ppm, in 3/10 females that received 5,000 ppm, and in 1/10 that received 2,500 ppm.

For mice, final mean body weights of the 5,000-ppm groups were 13% lower than that of the controls for males and 15% lower for females. Two of 10 male mice that

received 5,000 ppm and 1/10 males that received 300 ppm died before the end of the 13-week studies. Estimated feed consumption was similar for dosed and control groups. Degeneration of the germinal epithelium of the testis was observed in males that received 1,300 to 5,000 ppm; necrosis of the ovarian follicles was observed in females that received 5,000 ppm but not in the lower dose groups. Necrosis of the renal tubular epithelium was observed in 2/9 males that received 5,000 ppm.

Based on these results, 2-year studies of nitrofurantoin were conducted by feeding diets containing 0, 1,300, or 2,500 ppm nitrofurantoin to groups of 50 male F344/N rats and to groups of 50 male and female $B6C3F_1$ mice for 103 weeks. Groups of 50 female F344/N rats were fed diets containing 0, 600, or 1,300 ppm nitrofurantoin on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weight and average daily feed consumption of dosed male and female rats were similar to those of the controls throughout the studies. The average amount of nitrofurantoin consumed per day was estimated to be 60 and 110 mg/kg for low and high dose male rats and 30 and 60 mg/kg for low and high dose female rats. No significant differences in the number of rats surviving to the end of the studies were observed between any groups of rats of either sex (male: control, 24/50; low dose, 27/50; high dose, 26/50; female: 25/50; 26/50; 31/50).

Mean body weights of high dose male and female mice were up to 12% lower than those of the controls throughout most of the studies. The average daily feed consumption by dosed mice ranged from 93% to 100% that by controls. The average amount of nitrofurantoin consumed per day was estimated to be 280-300 mg/kg and 570-580 mg/kg for low and high dose mice. The survival of the control group of female mice was lower than that of the dosed groups (control, 19/50; low dose, 37/50; high dose, 37/50). The decrease in survival was most likely related to the increase in microbial infection in the reproductive tract observed in the controls. Groups of male mice had similar survival (28/50; 29/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Organs showing toxicity from nitrofurantoin exposure identified in the short-term studies were the testis in male rats and mice, the ovary in female rats and mice, and the kidney in male mice. Lesions observed in the 2- year studies were in the testis in male rats and mice, ovary in female mice, and kidney in male rats.

Chronic nephropathy was observed in nearly all rats, but the severity of the lesions was judged to be greater in dosed male rats. Hyperplasia of the transitional cell epithelium (control, 0/50; low dose, 5/50; high dose, 2/50) and hydronephrosis of the renal pelvis (0/50; 5/50; 2/50) were also observed in dosed male rats. In the standard single sections of the left and right kidney from each rat, tubular cell adenomas were observed in one low dose and two high dose males; a tubular cell carcinoma was observed in another high dose male. Because the number of renal tubular cell neoplasms identified by standard procedures in the dosed male rats was low, additional step-sections of the kidney were evaluated. The incidences of tubular cell adenomas derived from the step-sections and original sections (combined) were significantly increased in dosed male rats (adenomas: 3/50; 11/50; 19/50); tubular cell carcinomas occurred in two high dose males only.

Lesions considered to be associated with the nephropathy and nitrofurantoin exposure were observed in male rats and included hyperplasia of the parathyroid glands (3/49; 18/47; 23/49), fibrous osteodystrophy of the bone (0/50; 5/50; 5/50), and mineralization of the glandular stomach (1/49; 8/50; 14/50).

Atypical cells of the epididymis (0/50; 0/50; 12/50) and degeneration of the testis (0/50; 0/50; 36/50) were observed in high dose male rats. Fibrinoid necrosis of arterioles (1/50; 8/50; 15/50) and perivascular infiltration of mononuclear cells (3/50; 9/50; 19/50) were also observed in the testis of male rats. Interstitial cell adenomas of the testis occurred with a negative trend (47/50; 45/50; 21/50), and no adenomas or carcinomas of the preputial gland were seen in high dose male rats (12/48; 11/50; 0/47). The incidence of clitoral gland neoplasms was increased in low dose female rats (5/44; 10/38; 4/42).

Osteosarcomas were observed in the bone of one low dose and two high dose male rats. The historical incidence of osteosarcomas in untreated male F344/N rats is 8/1,937 (0.4%). The incidences of subcutaneous tissue neoplasms in dosed male rats were greater than that in the controls (1/50; 7/50; 5/50).

No neoplastic lesions in dosed female rats or male mice were considered to be compound related at the doses of nitrofurantoin administered.

For female mice, ovarian atrophy was observed in 48/50 low dose and 49/50 high dose mice but not in controls. Tubular cell adenomas of the ovary (0/50; 0/50; 5/50), benign mixed tumors (tubular and stromal) (0/50; 0/50; 4/50), and granulosa cell tumors (0/50; 3/50; 2/50)) were observed in dosed female mice. One granulosa cell tumor in the high dose group was malignant. Ovarian abscesses (18/50) and suppurative inflammation of the uterus (11/50) were observed in control female mice but not in dosed female mice and are believed to be related to indigenous microbial infections and most likely were the cause of early deaths in this group. Adenocarcinomas of the uterus were seen in one low dose and in one high dose mouse.

Testicular aspermatogenesis (1/49; 1/49; 16/50), degeneration of the germinal epithelium (0/49; 3/49; 23/50), and atypical cells (0/50; 0/49; 26/50) and depletion (1/50; 1/49; 15/50) of the epididymis were observed at increased incidences in high dose male mice.

Spindle cell hyperplasia of the adrenal cortex was observed in dosed female mice (3/50; 41/50; 45/50). A spindle cell adenoma (adrenal capsule adenoma) was seen in one low dose female mouse, and a spindle cell carcinoma (adrenal capsule carcinoma) was seen in one low dose male mouse.

Mineralization of the renal medulla (male: 0/50; 0/50; 17/50; female: 0/50; 0/50; 7/50) and dilatation of the renal tubules (male: 0/50; 0/50; 14/50) were observed in high dose mice.

Hepatocellular neoplasms (adenomas or carcinomas, combined) were observed at an increased incidence in high dose female mice (2/50; 2/50; 8/50). An Ito cell tumor of the liver was observed in one low dose and one high dose female mouse. Malignant lymphomas occurred in female mice (12/50; 19/50; 24/50).

Genetic Toxicology: Nitrofurantoin was mutagenic in *Salmonella typhimurium* strains TA98 and TA100, with and without metabolic activation, but was not mutagenic for strains TA1535 or TA1537. Nitrofurantoin induced forward mutations at the $TK^{+/-}$ locus of L5178Y mouse lymphoma cells in the absence of metabolic activation (it was not tested with activation). Nitrofurantoin induced increased numbers of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells with and without metabolic activation. Results of the sex-linked recessive lethal assay in Drosophila were negative after administration of nitrofurantoin by feeding or by injection.

Conclusions: Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity* of nitrofurantoin for male F344/N rats as shown by increased incidences of uncommon kidney tubular cell neoplasms. Uncommon osteosarcomas of the bone and neoplasms of the subcutaneous tissue were observed in dosed male rats. Incidences of interstitial cell adenomas of the testis and neoplasms of the preputial gland were decreased in the 2,500-ppm group of male rats. There was *no evidence of carcinogenic activity* of nitrofurantoin for female F344/N rats fed diets containing 600 ppm or 1,300 ppm for 2 years. Female rats may have been able to tolerate higher doses. There was *no evidence of carcinogenic activity* of nitrofurantoin for male $B6C3F_1$ mice fed diets containing 1,300 ppm or 2,500 ppm for 2 years. There was *clear evidence of carcinogenic activity* of nitrofurantoin for female $B6C3F_1$ mice as shown by increased incidences of tubular adenomas, benign mixed tumors, and granulosa cell tumors of the ovary.

Nonneoplastic lesions considered related to nitrofurantoin exposure were chronic nephropathy and associated lesions (hyperplasia of the parathyroid gland, fibrous osteodystrophy of the bone, and mineralization of the glandular stomach) in male rats and testicular degeneration in male rats and mice. Ovarian atrophy and hyperplasia of the adrenal cortex spindle cells were observed in dosed female mice.

Synonyms: 1-(((5-nitro-2-furanyl)methylene)amino-2,4-imidazolidinedione); 1-(5-nitro-2-furfurylideneamino)-hydantoin; *N*-(5-nitro-2-furfurlidene)-1-aminohydantoin; 1-((5-nitrofurfurylidene)amino)hydantoin

Trade Names: Benkfuran; Benkfurin; Chemiofuran; Cyantin; Dantafur; Furadantin; Furadantine; Furadantoin; Furadonin; Furadonine; Furantoin; Furatoin; Furobactina; Ituran; Macrodantin; Nifurantin; NSC 2107; N-Toin; Orafuran; Parafuran; Urizept; USAF EA-2; Welfurin; Zoofurin

Report Date: September 1989

TR-342 Toxicology and Carcinogenesis Studies of Dichlorvos (CAS No. 62-73-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of dichlorvos (99% pure), a contact and stomach poison for control of insects and parasites, were conducted by administering dichlorvos in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 13 weeks or 2 years. Previous feed studies were done by the National Cancer Institute using Osborne-Mendel rats and $B6C3F_1$ mice.

Thirteen-Week Studies: Thirteen-week studies with groups of 10 rats of each sex were conducted at doses of 0, 2, 4, 8, 16, 32, or 64 mg/kg dichlorvos in corn oil. All rats that received 32 or 64 mg/kg dichlorvos and 4/10 females that received 16 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control rats were similar. Thirteen-week studies with groups of 10 mice of each sex were conducted at doses of 0, 5, 10, 20, 40, 80, or 160 mg/kg. All 10 male mice and 9/10 female mice that received 160 mg/kg and 5/10 male mice that received 80 mg/kg dichlorvos died before the end of the studies. Final mean body weights of dosed and vehicle control mice were similar. No compound-related gross or microscopic pathologic effects were observed in rats or mice.

Two-year studies of dichlorvos were conducted by administering 0, 4, or 8 mg/kg dichlorvos, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex. Groups of 50 male $B6C3F_1$ mice were administered 0, 10, or 20 mg/kg dichlorvos on the same schedule, and groups of 50 $B6C3F_1$ female mice were administered 0, 20, or 40 mg/kg dichlorvos.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control rats and mice were similar. No significant differences in survival were observed between any groups of rats or mice of either sex (rats—male: vehicle control, 31/50; low dose, 25/50; high dose, 24/50; female: 31/50; 26/50; 26/50; mice—male: 35/50; 27/50; 29/50; female: 26/50; 29/50; 34/50).

Neoplastic Effects in the Two-Year Studies: Adenomas of the exocrine pancreas occurred at greater incidences in dosed rats than in vehicle controls (male: vehicle control, 25/50; low dose, 30/49; high dose, 33/50; female: 2/50; 3/47; 6/50). Mononuclear cell leukemia in both dosed groups of male rats occurred more frequently than in vehicle controls (11/50; 20/50; 21/50). Mammary gland fibroadenomas and fibroadenomas or adenomas (combined) in dosed female rats occurred at increased incidences relative to the vehicle controls (9/50; 19/50; 17/50). Multiple fibroadenomas occurred in dosed female rats but not in vehicle controls (0/50; 6/50; 3/50); carcinomas occurred in two vehicle control and two low dose female rats.

In mice, incidences of squamous cell papillomas of the forestomach were increased in the high dose groups compared with those in the vehicle controls (male: 1/50;

1/50; 5/50; female: 5/49; 6/49; 18/50). Two high dose female mice developed forestomach squamous cell carcinomas.

Genetic Toxicology: Dichlorvos was mutagenic in *Salmonella typhimurium* strain TA100 with and without metabolic activation but was not mutagenic in strain TA98. Dichlorvos was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay without metabolic activation. Dichlorvos induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells in the absence and presence of metabolic activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of dichlorvos for male F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mononuclear cell leukemia. There was *equivocal evidence of carcinogenic activity* of dichlorvos for female F344/N rats, as shown by increased incidences of adenomas of the exocrine pancreas and mammary gland fibroadenomas. There was *some evidence of carcinogenic activity* of dichlorvos for male B6C3F$_1$ mice, as shown by increased incidences of forestomach squamous cell papillomas. There was *clear evidence of carcinogenic activity* of dichlorvos for female B6C3F$_1$ mice, as shown by increased incidences of forestomach squamous cell papillomas.

Synonyms: 2,2-dichloroethenyl dimethyl phosphate; 2,2-dichlorovinyl dimethyl phosphate; *O,O*-dimethyl-*O*-(2,2-dichlorovinyl)phosphate; DDVP

Trade Names: BAY-19149; DDVF; ENT-20738; OMS-14; SD 1750; Canogard®; Crossman's Fly-Cake®; Dedevap®; De-Pester Insect Strip®; Estrosol®; Herkol®; Kill-fly Resin Strip®; Lethalaire®; Mafu®; Misect®; Nogos®; Nuvan®; No-Pest Strip®; Oko®; Phoracide®; Phosvit®; Vapona®; Vaponicide®; Vaporette Bar®

Anthelmintics: Atgard®; Dichlorman®; Equigard®; Task®

Report Date: September 1989

Note: Dichlorvos (technical grade) was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice administered in feed (See TR-10, reported 1979).

TR-343 Toxicology and Carcinogenesis Studies of Benzyl Alcohol (CAS No. 100-51-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years.

Short-Term Studies: In 16-day studies, all five male and five female rats and mice dosed with 2,000 mg/kg benzyl alcohol died. Two of five male and 3/5 female rats and 1/5 male and 2/5 female mice dosed with 1,000 mg/kg died. Rats and mice of each sex in the two highest dose groups were lethargic after dosing. Other toxic responses to benzyl alcohol in these dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts of rats and blood in the urinary bladder of mice. Animals administered lower doses of benzyl alcohol (125, 250, or 500 mg/kg) had no compound-related histologic lesions.

Doses selected for the 13-week studies were 0, 50, 100, 200, 400, and 800 mg/kg for rats and mice. Eight of 10 male rats dosed with 800 mg/kg died during weeks 7 and 8; four of these deaths were described as gavage related. Rats dosed with 800 mg/kg exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. In mice, deaths were scattered among all dose levels, but none occurred in vehicle controls. Four male and six female mice died after being dosed; all deaths but one were described as gavage related. Staggering after dosing also occurred during the first 2 weeks of the studies in mice dosed with 800 mg/kg. Some of the deaths in the rats and mice may have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. There were reductions in relative weight gain in male rats dosed with 800 mg/kg benzyl alcohol, in female rats dosed with 200 mg/kg or more, in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in body weight gain or compound-related histopathologic lesions were observed in rats or mice from the lower dose groups. Based on mortality, reduction in relative body weight gain, and the histopathologic lesions, doses selected for 2-year studies in rats were 0, 200, and 400 mg/kg. Doses selected for 2-year studies in mice were 0, 100, and 200 mg/kg, based on mortality and depression in relative body weight gain.

Body Weight and Survival in the Two-Year Studies: Fifty animals of each species and sex were administered benzyl alcohol in corn oil by gavage 5 days per week for 103 weeks. Administration of benzyl alcohol did not affect survival in male rats (final survival rates: vehicle control, 28/50; low dose, 27/50; high dose, 24/50) but reduced survival of dosed female rats by half (36/50; 18/50; 17/50). Many of the early deaths were considered related to the gavage procedure. Survival in mice was not affected by benzyl alcohol administration (male: 34/50; 33/50; 35/50; female: 26/50; 32/50; 36/50). No effect of benzyl alcohol on body weight gain in rats or mice was observed. In the third month of the studies, clinical signs of sialodacryoadenitis virus infection were observed in rats. A positive serologic reaction for rat coronavirus was observed in sentinel animals at 6 months and again at 18 months.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No apparent compound-related nonneoplastic responses were observed. Dose-related negative trends

in the incidences of anterior pituitary gland neoplasms were seen in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Adenomas of the adrenal cortex occurred at an increased incidence in high dose male mice (0/48; 0/44; 3/48), but this slight increase was not considered to be related to chemical exposure.

Genetic Toxicology: Benzyl alcohol was not mutagenic when tested by the preincubational protocol in the presence or absence of exogenous metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537. In the mouse L5178Y/TK$^{+/-}$ lymphoma assay, benzyl alcohol induced an increase in trifluorothymidine (Tft)-resistant cells in the absence, but not in the presence, of S9; the effect was associated with toxicity. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with benzyl alcohol produced an increase in sister chromatid exchanges (SCEs) which was judged to be equivocal both with and without S9; a significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not the absence, of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of benzyl alcohol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg/kg. Survival in both dose groups of female rats was 50% that of vehicle controls, primarily due to an increased number of gavage-related deaths. There was *no evidence of carcinogenic activity* of benzyl alcohol for male or female B6C3F$_1$ mice dosed with 100 or 200 mg/kg for 2 years.

Synonyms: benzenemethanol; phenylcarbinol; phenylmethanol; α-hydroxytoluene; benzenecarbinol; phenolcarbinol; α-toluenol

Report Date: June 1989

TR-344 Toxicology and Carcinogenesis Studies of Tetracycline Hydrochloride (CAS No. 64-75-5) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Tetracycline hydrochloride is a broad-spectrum antibiotic used for its bactericidal action in human and veterinary medicine. Toxicology and carcinogenesis studies of tetracycline hydrochloride (USP grade, 91% pure) were conducted by feeding diets containing tetracycline hydrochloride to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: The same dietary concentrations were used for the 14-day and 13-week studies (0, 3,125, 6,250, 12,500, 25,000 and 50,000 ppm tetracycline hydrochloride). In the 14-day studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 24% lower than that of the controls. The final mean body weight of mice that received 50,000 ppm in the diet was 18% lower than that of the controls for males and 15% lower for females. No compound-related effects were observed in rats or mice at necropsy.

During the 13-week studies, none of the rats or mice died. The final mean body weight of male rats that received 50,000 ppm was 18% lower than that of the controls. Compound-related effects included cytoplasmic vacuolization in the liver of male rats at 25,000 and 50,000 ppm. Bone tetracycline concentrations in rats and mice increased with increasing dose of tetracycline hydrochloride. The final mean body weight of mice that received 50,000 ppm was 16% lower than that of the controls for males and 6% lower for females. Estimated feed consumption by dosed rat and mouse groups was similar to that of the controls. No compound-related gross or microscopic pathologic effects were observed in mice.

Based on these results, 2-year studies of tetracycline hydrochloride were conducted by feeding diets containing 0, 12,500, or 25,000 ppm tetracycline hydrochloride to groups of 50 rats and 50 mice of each sex for 103 weeks.

Body Weight, Survival, and Feed Consumption in the Two-Year Studies: Mean body weights of dosed and control male and female rats were similar throughout most of the studies. The survival of both the low and high dose female groups was greater than that of the controls. No significant differences in survival were observed between any groups of male rats (male: control, 27/50; low dose, 24/50; high dose, 31/50; female: 27/50; 39/50; 38/50). Mean body weights of dosed mice were markedly (more than 10%) lower than those of the controls throughout most of the studies. The survival rates of the dosed groups of male mice were greater than that of the control group. No significant differences in survival were observed between any groups of female mice (male: 31/50; 43/50; 43/50; female: 37/50; 35/50; 38/50). Feed consumption was similar by dosed and control rats and mice of either sex throughout the studies.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Basophilic cytoplasmic change and clear cell change were positively correlated with tetracycline hydrochloride administration in male rats. Otherwise, no significant increases in neoplastic or nonneoplastic lesions in rats or mice of either sex were considered related to tetracycline hydrochloride administration.

The incidence of adenomas or carcinomas (combined) of the pancreatic islets in low dose male rats was greater than that in the controls (control, 0/49; low dose, 5/49; high dose, 0/49). This marginal effect in the low dose group was not considered to be chemically related. The historical control rate of pancreatic islet cell neoplasms from previous studies at this laboratory is 6% (9/148).

Decreased incidences and severity of chronic nephropathy in male rats were associated with tetracycline hydrochloride administration (48/50; 35/50; 36/50). Female mice administered tetracycline hydro-

chloride in feed did not develop hepatocellular adenomas or carcinomas (combined incidence: 10/49; 0/48; 0/50). The historical control rate for hepatocellular adenomas or carcinomas (combined) from previous studies at this laboratory is 18/149 (12%). Other decreases in tumor incidence involving several tissues were considered to be of marginal biologic significance.

Genetic Toxicology: Tetracycline hydrochloride was not mutagenic in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, or TA1537) when tested in a preincubation protocol in the presence or absence of exogenous metabolic activation. Tetracycline hydrochloride was negative in the mouse lymphoma L5178Y/TK$^{+/-}$ assay with or without induced rat liver S9 but gave a marginally positive response when tested in the presence of noninduced S9. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with tetracycline hydrochloride, both with and without S9, did not induce chromosomal aberrations or sister chromatid exchanges (SCEs). Tetracycline hydrochloride did not induce sex-linked recessive lethal mutations when administered by feeding or injection to adult male Drosophila.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of tetracycline hydrochloride for male or female F344/N rats and B6C3F$_1$ mice fed diets containing 12,500 or 25,000 ppm. Tetracycline hydrochloride-dosed female rats and male mice had greater survival rates than the respective controls during these studies. Dosed mice had lower body weight than controls, and dosed female mice had no hepatocellular adenomas or carcinomas.

Trade Names for Tetracycline or Tetracycline hydrochloride: Achromycin; Amycin; Bristacycline; Cyclopar; Dumocyclin; Neocyclin B; Panmycin; Polycycline; Robitet; Ro-cycline; Steclin; Sumycin; Topicycline; Unimycin

Report Date: August 1989

TR-345 Toxicology and Carcinogenesis Studies of Roxarsone (CAS No. 121-19-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Roxarsone is a veterinary drug used as a growth promoter and as an anticoccidial agent and for treatment of swine dysentery. Toxicology and carcinogenesis studies were conducted by administering roxarsone (greater than 99.4% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, the diets fed to rats contained 0 or 100-1,600 ppm roxarsone, and those fed to mice contained 0 or 60-1,000 ppm. Deaths occurred in rats and mice that received the highest doses. Rats that received 800 or 1,600 ppm lost weight. Male mice that received 1,000 ppm and female mice that received 500 ppm lost weight.

In the first 13-week studies, roxarsone was fed to rats and mice at dietary concentrations of 0 or 50-800 ppm. Decreases (more than 10%) in final mean body weights of dosed rats relative to those of controls were observed for males that received 200, 400, or 800 ppm and for females that received 400 or 800 ppm. Deaths occurred in groups that received 800 ppm. Clinical signs of toxicity (trembling, ataxia, and pale skin) were seen primarily in rats that received 800 ppm. Kidney lesions were observed in rats that received 800 ppm. These lesions were characterized by tubular necrosis and mineralization in the rats that died during the studies and by tubular dilatation and casts, interstitial inflammation, and tubular epithelial cell regeneration in the rats that lived to the end of the studies.

Additional 13-week studies were conducted in rats at dietary concentrations of 0, 100, or 400 ppm to demonstrate the absorption of roxarsone from the gastrointestinal tract; to determine its distribution in liver, kidney, and blood; and to study its effects on various hematologic and clinical chemical values. No deaths occurred. Renal lesions of minimal severity observed in male rats that received 400 ppm were characterized by tubular epithelial cell degeneration and regeneration, tubular casts, and mineralization. Arsenic levels in urine, blood, kidney, and liver of dosed rats increased (140%-300%) with time on study and were proportional to the dietary concentrations of roxarsone. No compound-related hematologic or clinical chemical effects were observed in rats.

In the first 13-week studies, final mean body weights of mice that received 800 ppm were 11%-18% lower than those of controls. Deaths occurred in males and females receiving 400 and 800 ppm. No compound-related gross or histopathologic lesions were observed.

In the second 13-week studies in mice, no compound-related hematologic or clinical chemical effects were observed. At the end of the studies, arsenic concentrations in dosed mice ranged from 0.45 to 0.99 μg/g of liver and from 0.85 to 2.98 μg/g of kidney. No arsenic was detected in the liver or kidney of control mice.

Because of kidney lesions, lower body weight gain, and increased mortality in rats and lower body weight gain and increased mortality in mice in the short-term studies, dietary concentrations of roxarsone selected for the 2-year studies were 0, 50, or 100 ppm for rats and 0, 100, or 200 ppm for mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally within 5% of those of controls. No significant differences in survival were observed between any groups of rats of either sex, although survival in males was lower than usual (final survival—male: control, 24/50; low dose, 18/50; high dose, 18/50; female: 27/50; 35/50; 32/50). The average feed consumption by high dose rats was 95% that of controls for males and 88% for females. The average amount of roxarsone consumed per day was approximately 2 mg/kg for low dose rats and 4 mg/kg for high dose rats. Mean body weights of high dose male mice were generally 5%-8% higher than those of the

controls, whereas those of female mice were generally 6%-15% lower than those of the controls. The survival of the control group of male mice was lower than that of the low dose group after month 22; survival for females was low (final survival—male: 27/50; 40/50; 33/50; female: 14/50; 18/50; 17/50). The low survival in females was due in part to utero-ovarian infection, with more than 50% of the animals in each dose group having suppurative inflammation at this site. The average daily feed consumption by dosed mice was 105%-110% that by the controls. The average amount of roxarsone consumed per day was approximately 21 or 43 mg/kg for low dose or high dose male mice and 27 or 54 mg/kg for low dose or high dose female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Although the incidence of adenomas of the exocrine pancreas in high dose male rats was not statistically greater than that in the controls (control, 1/50; low dose, 1/50; high dose, 5/50), it was greater than that seen in any historical control group of male F344/N rats. The historical rate is 1/437 (0.2%) for the study laboratory and 5/1,871 (0.3%) throughout the Program. The incidences of hyperplasia were 2/50; 0/50; 3/50. No hyperplasia or adenomas were observed in the exocrine pancreas of female rats.

Clitoral gland adenomas in female rats occurred with a marginally positive trend (1/44; 3/47; 6/48; $P = 0.049$). One carcinoma was also observed in each of the groups. The incidences of adenomas or of adenomas or carcinomas (combined) in the dosed groups were not significantly different from those in the controls. This marginal effect was not considered to be related to roxarsone administration.

No chemical-related increases in neoplastic or nonneoplastic lesions occurred in male or female mice. Lymphomas in female mice occurred with a negative trend; the incidences in the dosed groups were lower than that in the controls (13/50; 2/50; 3/50; $P \leq 0.01$).

Genetic Toxicology: Roxarsone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Roxarsone induced trifluorothymidine (Tft) resistance in mouse lymphoma L5178Y cells in the absence of metabolic activation; it was not tested with activation. Exposure of adult male *Drosophila melanogaster* to roxarsone by injection or by feeding did not cause an increase in sex—linked recessive lethal mutations.

Audit: The data, documents, and pathology materials from the 2-year studies of roxarsone have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of roxarsone for male F344/N rats, as indicated by a marginally increased incidence of adenomas of the exocrine pancreas. There was *no evidence of carcinogenic activity* for female F344/N rats fed diets containing 50 or 100 ppm roxarsone for 2 years. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice fed diets containing 100 or 200 ppm roxarsone for 2 years.

Synonyms: 4-hydroxy-3-nitrophenylarsonic acid; 4-hydroxy-3-nitrobenzenearsonic acid; 2-nitro-1-hydroxybenzene-4-arsonic acid; nitrophenolarsonic acid; 3-nitro-4-hydroxybenzenearsonic acid; 3-nitro-4-hydroxyphenylarsonic acid

Trade Names: Ristat; Ren-O-sal; 3-nitro; 3-nitro-10; 3-nitro-20; 3-nitro-50; 3-nitro-80

Report Date: March 1989

TR-346 Toxicology and Carcinogenesis Studies of Chloroethane (Ethyl Chloride) (CAS No. 75-00-3) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Toxicology and carcinogenesis studies of chloroethane (99.5% pure), an alkylating agent and chemical intermediate, as well as a topical and inhalation anesthetic, were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to chloroethane by whole-body inhalation once for 4 hours or for 6 hours per day, 5 days per week for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

Single-Exposure, Fourteen-Day, and Thirteen-Week Studies: In the single-exposure and 14-day inhalation studies, all rats and mice exposed to 19,000 ppm chloroethane survived. The animals were not exposed at lower concentrations. No clinical signs of toxicity were seen. In the 14-day studies, final mean body weights of exposed male rats and exposed mice were higher than those of controls. Mean body weights of exposed and control female rats were similar.

In the 13-week studies, rats and mice were exposed to 0, 2,500, 5,000, 10,000, or 19,000 ppm chloroethane. No compound-related deaths occurred in rats or mice. The final mean body weight of rats exposed to 19,000 ppm was 8% lower than that of controls for males and 4% lower for females. Final mean body weights of exposed mice were generally higher than those of controls. No compound-related clinical signs or gross or microscopic pathologic effects were seen in rats or mice. The liver weight to body weight ratios for male rats and female mice exposed to 19,000 ppm were greater than those for controls. Although no chemically related toxic effects were observed in the short-term studies, concerns about potential flammability and explosion led to the selection of 0 and 15,000 ppm as the exposure concentrations for rats and mice for the 2-year studies.

Body Weight and Survival in the Two-Year Studies: Mean body weights of exposed male rats were 4%-8% lower than those of controls after week 33. Mean body weights of exposed female rats were generally 5%-13% lower than those of controls throughout the study. Although survival of male rats and exposed female rats was low at the end of the studies (male: control, 16/50;

exposed, 8/50; female: 31/50; 22/50), no statistically significant differences in survival were observed between exposed and control groups of either sex. Survival at week 90 for male rats was 37/50 (control) and 31/50 (exposed) and for females, 43/50 (control) and 33/50 (exposed). The high incidence of mononuclear cell leukemia may have contributed to the high mortality.

Mean body weights of exposed male mice were up to 13% higher than those of controls throughout the study. Mean body weights of exposed and control female mice were generally similar throughout the study. The survival of the exposed groups of both male (after day 330) and female (after day 574) mice was significantly lower than that of controls (final survival – male: 28/50; 11/50; female: 32/50; 2/50). The majority of exposed female mice died as a result of uterine carcinomas. Male mice, and particularly exposed mice, died early as a result of an ascending urinary tract infection.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Malignant astrocytomas of the brain were seen in three exposed female rats, and gliosis was observed in a fourth. The historical incidence of glial cell neoplasms in untreated control female F344/N rats is 23/1,969. The highest incidence observed in an untreated control group is 3/50.

Trichoepitheliomas (1/50), sebaceous gland adenomas (1/50), basal cell carcinomas (3/50), and squamous cell carcinomas (2/50) of the skin were observed only in exposed male rats. Keratoacanthomas occurred in four control and two exposed male rats.

Exposure of female mice to chloroethane caused a high incidence of uterine carcinomas of endometrial origin (control, 0/49; exposed, 43/50). One control female did have a uterine carcinoma, although it was not of endometrial origin. The tumors observed in 34 exposed females were highly malignant, invading the uterine myometrium and metastasizing to a wide variety of organs, primarily lung (23), ovary (22), lymph nodes (18), kidney (8), adrenal gland (8), pancreas (7), mesentery (7), urinary bladder (7), spleen (5), and heart (4), and to a lesser extent, colon, stomach, gallbladder, small intestine, ureter, and liver.

Two marginally increased incidences of other neoplasms were observed in exposed male and female mice. The incidence of hepatocellular carcinomas in exposed female mice was greater than that in controls (3/49; 7/48). One other exposed female had a hepatocellular adenoma. The incidence of alveolar/bronchiolar neoplasms of the lung in exposed male mice was greater than that in controls (adenomas or carcinomas, combined: 5/50; 10/48).

Genetic Toxicology: Chloroethane, tested within the closed environment of a desiccator, was mutagenic with and without exogenous metabolic activation in *S. typhimurium* strain TA1535; in strain TA100, a positive response was observed only with activation. No mutagenic activity was observed in *S. typhimurium* strain TA98 with or without metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity* of chloroethane for male F344/N rats, as indicated by benign and malignant epithelial neoplasms of the skin. For female F344/N rats, there was *equivocal evidence of carcinogenic activity*, as indicated by three uncommon malignant astrocytomas of the brain in the exposed group. The study of male $B6C3F_1$ mice was considered to be an *inadequate study of carcinogenicity* because of reduced survival in the exposed group. However, there was an increased incidence of alveolar/bronchiolar neoplasms of the lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, as indicated by carcinomas of the uterus. A marginally increased incidence of hepatocellular neoplasms was observed in the exposed group.

Synonyms: monochloroethane; chloroethyl; ether hydrochloric; ether muriatic; aethylis; aethylis chloridum; ether chloridum; ether chloratus

Trade Names: Kelene; Chelen; Anodynon; Chloryl Anesthetic; Narcotile

Report Date: October 1989

TR-347 Toxicology and Carcinogenesis Studies of *d*-Limonene (CAS No. 5989-27-5) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of *d*-limonene, a naturally occurring monoterpene found in many volatile oils, especially in citrus oils, were conducted because of its widespread use as a flavor and fragrance additive for food and household cleaning products and its increasing use as an industrial solvent. The *d*-limonene used in these studies was more than 99% pure and was administered in corn oil by gavage. Short-term studies were conducted in F344/N rats and $B6C3F_1$ mice to identify toxic effects and affected sites and to help establish doses for the 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

The doses selected for the 16-day studies ranged from 413 to 6,600 mg/kg for both rats and mice; deaths and reduction in body weight gain occurred at the two highest doses. No compound-related clinical signs or histopathologic lesions were observed in any of the surviving dose groups.

In the 13-week studies, doses of *d*-limonene ranged from 150 to 2,400 mg/kg for rats and from 125 to 2,000 mg/kg for mice. Deaths occurred in the high dose group of each species and sex. Greater than 10% reductions in body weight gain were observed in the two highest dose groups of male rats and male mice and the high dose female rats. Rough hair coats and decreased activity were observed at the two highest doses in both rats and mice. There were no chemical-related histopathologic lesions in female rats or in mice of either sex. A compound-related increased severity of nephropathy was observed in the kidney of male rats. This lesion was

characterized by degeneration of epithelial cells in the convoluted tubules, granular casts in the outer stripe of the outer medulla, and epithelial regeneration. These lesions have been described as reasonably characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_2\mu$-globulin in the cytoplasm of tubular epithelial cells.

Two-year studies of *d*-limonene were conducted by administering 0, 75, or 150 mg/kg *d*-limonene in corn oil by gavage to groups of 50 F344/N male rats, 5 days per week for 103 weeks; groups of 50 female F344/N rats were administered 0, 300, or 600 mg/kg. These doses were selected based on compound-related, potentially life-threatening kidney lesions observed in males at 300 mg/kg and higher and on the large number of deaths of female rats at 2,400 mg/kg. Groups of 50 male $B6C3F_1$ mice were administered 0, 250, or 500 mg/kg according to the same schedule; groups of 50 female $B6C3F_1$ mice were administered 0, 500, or 1,000 mg/kg. These doses were selected based on the deaths observed for both male and female mice at 2,000 mg/kg during the 13-week studies and the body weight depression in male mice at 1,000 mg/kg and higher.

Mean body weights of rats dosed with *d*-limonene were similar to those of vehicle controls throughout the studies. Survival of the high dose female rats after week 39 and of the vehicle control male rats after week 81 was significantly reduced (survival at week 104 – male: vehicle control, 29/50; low dose, 33/50; high dose, 40/50; female: 42/50; 40/50; 26/50). Mean body weights of dosed and vehicle control male mice were similar throughout the studies. Mean body weights of high dose female mice were notably lower than those of the vehicle controls after week 28. Survival of the low dose group of male mice was significantly lower than that of vehicle controls at the end of the study (33/50; 24/50; 39/50). No difference in survival was observed between vehicle control and dosed female mice (43/50; 44/50; 43/50).

In the 2-year studies, the kidney was confirmed as the primary target organ for chemically related lesions. No lesions were observed in female rats. For males, the nonneoplastic lesions included exacerbation of the age-related nephropathy, linear deposits of mineral in the renal medulla and papilla, and focal hyperplasia of the transitional epithelium overlying the renal papilla. Uncommon tubular cell adenomas and adenocarcinomas of the kidney also occurred in dosed male rats, and this effect was supported by a dose-related increased incidence of tubular cell hyperplasia, as shown in the table below (see page 4 of the Technical Report).

In subsequent 21-day studies, male and female F344/N rats were administered *d*-limonene at doses ranging from 75 to 1,200 mg/kg. Microscopic examination of the kidney sections from these rats indicated a compound-related increase in intracytoplasmic granules in the proximal convoluted tubules of dosed male rats but not of female rats. The granules were shown to contain $\alpha_2\mu$-globulin by an immunohistochemical strain. $\alpha_2\mu$-Globulin was shown to be increased in kidney homogenates from dosed male rats by an ELISA test.

In mice, no chemically related increases in neoplasms were observed. The incidence of neoplasms of the anterior pituitary gland in high dose female mice was lower than that in vehicle controls (adenomas or carcinomas, combined: vehicle control, 12/49; high dose, 2/48). Cells with an abnormal number of nuclei (8/49; 32/50) and cytomegaly (23/49; 38/50) were observed in the liver of high dose male mice.

Genetic Toxicology: *d*-Limonene was not mutagenic in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537), did not significantly increase the number of trifluorothymidine (Tft)-resistant cells in the mouse L5178Y/TK$^{+/-}$ assay, and did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of *d*-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was *no evidence of carcinogenic activity* of *d*-limonene for female F344/N rats that received 300 or 600 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for male $B6C3F_1$ mice that received 250 or 500 mg/kg. There was *no evidence of carcinogenic activity* of *d*-limonene for female $B6C3F_1$ mice that received 500 or 1,000 mg/kg.

An increased severity of spontaneous nephropathy, increased incidences of linear mineralization of the renal medulla and papilla, and hyperplasia of the transitional epithelium of the renal papilla were present in dosed male rats.

Synonyms: cyclohexene; 4-isopropenyl-1-methyl; 1-methyl-4-(1-methylethenyl)cyclohexene; *p*-mentha-1,8-diene; carvene; cinene; cajeputene

Report Date: January 1990

TR-348 Toxicology and Carcinogenesis Studies of *alpha*-Methyldopa Sesquihydrate (CAS No. 41372-08-1) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

α-Methyldopa sesquihydrate is used in the treatment of hypertension; over 20 million prescriptions are written annually for α-methyldopa or α-methyldopa sesquihydrate in the United States. α-Methyldopa sesquihydrate (USP grade, greater than 99% pure) was selected for study because of widespread human exposure and the lack of carcinogenicity studies on this compound.

Fourteen-day, 13-week, and 2-year studies were conducted in F344/N rats and $B6C3F_1$ mice. The chemical was administered in feed because human exposure is primarily by the oral route. Short-term studies were performed in bacteria and mammalian cells to evaluate the potential for genetic damage.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, the chemical was administered at dietary concentrations of 0 and 6,250-100,000 ppm. All rats receiving 100,000 ppm and 2/5 female rats receiving 50,000 ppm died. All mice lived until the end of the studies. Final mean body weights of dosed male rats were 14%-43% lower than that of controls, and those of dosed female rats were 9%-24% lower. Feed consumption by dosed male and female rats was reduced. Final mean body weights of dosed mice were generally within 10% of those of controls; feed consumption by dosed groups was lower than that by controls during the first week of the studies.

In the 13-week studies, the chemical was administered at dietary concentrations of 0 and 3,100-50,000 ppm. Deaths occurred in 4/10 male rats, 7/10 female rats, and 2/10 female mice at 50,000 ppm and in 1/10 female rats at 25,000 ppm. Final mean body weights of dosed rats were 6%-46% lower than those of controls. Feed consumption by dosed rat groups was lower than that by controls. Final mean body weights of male mice at 25,000 and 50,000 ppm and female mice at 50,000 ppm were reduced 12%-19%. Feed consumption by dosed and control mice was comparable.

Rats and mice receiving 25,000 and 50,000 ppm exhibited clinical signs of toxicity including lethargy, hyperexcitability, ocular discharge, and rough hair coats. Clinical signs of toxicity were judged to be more severe in dosed male mice than in female mice. Minimal to moderate kidney tubular cell regeneration was seen in male and female rats at 12,500, 25,000, and 50,000 ppm. Bone marrow hypoplasia occurred in male rats at 25,000 and 50,000 ppm and in female rats at 6,300 ppm and higher. Nuclear enlargement (karyomegaly) of the renal cortical tubular epithelium was observed in male and female mice administered 12,500-50,000 ppm; these kidney lesions were judged to be more severe and occurred more frequently at concentrations of 25,000 ppm and higher.

Because of kidney lesions, bone marrow responses, and body weight effects at 12,500 ppm and higher and increased deaths and clinical signs at 25,000 and 50,000 ppm, dietary concentrations selected for male and female rats in the 2-year studies were 0, 3,100, and 6,300 ppm. Based on clinical signs, kidney effects, and body weight decreases at 25,000 and 50,000 ppm, dietary concentrations selected for male and female mice in the 2-year studies were 0, 6,300, and 12,500 ppm. Diets containing the chemical at these concentrations were fed to groups of 50 male and 50 female rats and 50 male and 50 female mice for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally 8%-17% lower than those of controls, and mean body weights of dosed mice were generally 5%-22% lower than those of controls throughout the studies. The average amount of α-methyldopa sesquihydrate consumed per day was approximately 110-120 or 230-240 mg/kg per day by low and high dose rats and 830-890 or 1,760-1,800 mg/kg by low and high dose mice. Survival was comparable among dosed and control groups (male rats: control, 28/50; low dose, 26/50; high dose, 27/50; female rats: 35/50; 34/50; 29/50; male mice: 44/50; 42/50; 39/50; female mice: 42/50; 40/50; 38/50). Clinical signs considered to be dose-related included fighting in male rats, irritability in male mice, and rough hair coats in female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Several lesions of the forestomach, including edema, chronic inflammation, epithelial hyperplasia, and ulcers, were seen at low incidences in high dose rats. No forestomach neoplasms occurred. No neoplastic lesions were observed in either male or female rats which were considered related to α-methyldopa sesquihydrate exposure.

Nephropathy (control, 3/50; low dose, 21/50; high dose, 32/50), karyomegaly (nuclear enlargement) of cells of the tubular epithelium (0/50; 46/50; 44/50, and cysts (2/50; 10/50; 10/50) were observed in the kidney of dosed female mice. Low incidences of tubular cell hyperplasia (0/50; 1/50; 1/50), tubular cell adenomas (0/50; 2/50; 0/50), and tubular cell adenocarcinomas (0/50; 0/50; 1/50) were observed in male mice. Tubular cell adenomas (3/2,029, 0.15%) and tubular cell adenocarcinomas (3/2,029, 0.15%) are uncommon in untreated control male $B6C3F_1$ mice. No neoplastic lesions in female mice were considered related to α-methyldopa sesquihydrate exposure.

Decreased incidences of several site-specific neoplasms were observed in dosed rats and mice; these decreases might have been due in part to decreased weight gain in dosed groups. The decreases occurred in the adrenal medulla of male rats (pheochromocytomas or malignant pheochromocytomas, combined: 21/49; 3/49; 10/50), uterus of female rats (endometrial stromal polyps: 15/50; 5/49; 1/50), liver of male and female mice (hepatocellular adenomas or carcinomas, combined – male: 15/50; 5/50; 6/50; female: 4/50; 1/50; 0/50), and anterior pituitary gland of female mice (adenoma: 9/49; 4/40; 2/50). The incidences of malignant tumors (male: 19/50; 9/50; 8/50; female: 21/50; 16/50; 12/50) and benign or malignant tumors (combined) (male: 32/50; 15/50; 17/50; female: 33/50; 22/50; 21/50) were reduced in dosed mice.

Reproductive Studies: α-Methyldopa sesquihydrate was administered to male F344/N rats in corn oil by gavage 5 days per week for 65 days at doses of 0, 50, 100, 200, or 400 mg/kg. Decreased body weight was seen in dosed animals. Male rats were mated to untreated female F344/N rats on days 57-61, necropsies were performed on days 65-67, and reproductive toxicity was measured by sperm count, sperm motility, organ weights, hormone levels, and histologic evaluation of the testis. Decreased fertility was observed in males dosed with α-methyldopa sesquihydrate at 200 and 400 mg/kg. Decreases were also seen in sperm count, sperm motility, apparent number of late spermatids, and plasma testosterone levels in males in the 200 and 400 mg/kg groups. This alteration of reproductive function in male rats was found to be reversible after a 13-week recovery period (without dosing). The decreased fertility observed after α-methyldopa sesquihydrate administration was probably due in part to the decreases in plasma testosterone levels.

Genetic Toxicity: α-Methyldopa sesquihydrate was not mutagenic when tested with or without exogenous metabolic activation with a preincubation protocol in four strains of *Salmonella typhimurium* (TA97, TA98, TA100, or TA1535). No increase in chromosomal aberrations or sister chromatid exchanges was observed in Chinese hamster ovary (CHO) cells exposed to α-methyldopa sesquihydrate with or without S9.

Audit: The data, documents, and pathology materials from the 2-year studies of α-methyldopa sesquihydrate have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of α-methyldopa sesquihydrate for male or female F344/N rats fed diets containing 3,100 or 6,300 ppm. There was *equivocal evidence of carcinogenic activity* of α-methyldopa sesquihydrate for male $B6C3F_1$ mice, as shown by three dosed mice having uncommon tubular cell tumors of the kidney. There was *no evidence of carcinogenic activity* of α-methyldopa sesquihydrate for female $B6C3F_1$ mice fed diets containing 6,300 or 12,500 ppm. Nonneoplastic lesions of the kidney including karyomegaly were observed in dosed female mice.

Decreased incidences of several tumor types (in the adrenal gland in male rats, uterus in female rats, liver in male and female mice, and anterior pituitary gland in female mice) were considered related to α-methyldopa sesquihydrate exposure.

Synonyms for α-Methyldopa or α-Methyldopa sesquihydrate: 3-hydroxy-α-methyl-L-tyrosine sesquihydrate; L-(α-MD); α-methyl-L-3,4-dihydroxyphenylalanine; L(-) - β - (3,4-dihydroxyphenyl) - α - methylalanine; L-(-)-3-(3,4-dihydroxyphenyl)-2-methylalanine; L-α-methyl-3,4-dihydroxyphenylalanine; α-methyl-β-(3,4-dihydroxyphenyl)-L-alanine; L-(-)-α-methyl-β-(3,4-dihydroxyphenyl)alanine; (-)-methyldopa; L-methyldopa; L-α-methyldopa; α-methyl-L-dopa

Trade Names for α-Methyldopa or α-Methyldopa sesquihydrate: Aldomet; Aldometil; Aldomin; α-Medopa; AMD; Bayer 1440 L; Baypresol; Dopamet; Dopatec; Dopegyt; Hyperpax; Medomet; Medopren; Methoplain; MK. B51; MK-351; Presinol; Presolisin; Sedometil; Sembrina

Report Date: March 1989

TR-349 Toxicology and Carcinogenesis Studies of Two Pentachlorophenol Technical-Grade Mixtures (CAS No. 87-86-5) in $B6C3F_1$ Mice (Feed Studies)

Toxicology studies of pentachlorophenol, a biocide used primarily as a wood preservative, were conducted by feeding diets containing a technical-grade composite, Dowicide EC-7 (a technical grade formulation), or pure pentachlorophenol to groups of $B6C3F_1$ mice for 30 days. These three grades plus another commercial grade of pentachlorophenol (DP-2) were used in 6-month studies. These studies were followed by 2-year carcinogenicity studies of technical-grade pentachlorophenol and of Dowicide EC-7 in feed. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary (CHO) cells.

Thirty-Day and Sixteen-Month Studies: Groups of 19 male mice and 5-15 female mice were fed diets containing 0, 20, 100, 500, 2,500, or 12,500 ppm technical-grade pentachlorophenol, Dowicide EC-7, or pure pentachlorophenol for 30 consecutive days. Necropsies and histopathologic examinations were performed on all animals. Selected organs were weighed. Supplemental analyses included hematology, serum chemistry, urinalysis, immunology, and hepatic enzyme induction. Compound-related deaths were observed at the highest dose (12,500 ppm) with all three materials and at 2,500 ppm with EC-7 and pure pentachlorophenol (males only). Decreases in body weight gain were also observed in the groups in which deaths occurred. Diffuse centrilobular cytomegaly, karyomegaly, nuclear atypia, degeneration, or necrosis of the liver were compound-related lesions observed in all groups that received pure pentachlorophenol, technical-grade pentachlorophenol, or EC-7 at 500 ppm and above. Serum enzymes associated with liver injury were increased.

In the 6-month studies, groups of 10 male and 10 female mice were given diets containing the various grades of pentachlorophenol at the following dietary concentrations: 200, 600, or 1,800 ppm technical-grade pentachlorophenol; 200, 600, or 1,200 ppm DP-2 (not used in the 30-day studies); 200, 600, or 1,200 ppm EC-7; or 200, 500, or 1,500 ppm pure pentachlorophenol for 26-27 weeks. Common control groups of 10 male and 10 female mice were fed control diets. Additional groups of male mice were examined for behavioral, histopathologic, clinical pathology, biochemical, and immunologic effects.

All mice exposed at the highest dose of technical-grade pentachlorophenol died, as did 2/10 male mice exposed at the highest dose of DP-2. No deaths were observed in mice exposed to EC-7 or pure pentachlorophenol. Markedly lower final body weights were observed in the high dose groups only (all grades of pentachlorophenol). No chemical-related clinical signs were observed at sublethal doses. No major behavioral changes were observed after 5 weeks' exposure, but increased motor activity and heightened startle responses were present at the end of the study in female mice exposed to all four grades of pentachlorophenol. All grades of pentachlorophenol caused increases in serum enzymes associated with liver injury. All grades of pentachlorophenol also resulted in a dose-related induction of aryl hydrocarbon hydroxylase and an increase in cytochrome P450. However, the technical grade was a more powerful inducer than the other grades of pentachlorophenol. Pure pentachlorophenol had no effect on humoral or cell-mediated immunity. However, DP-2 and particularly technical-grade pentachlorophenol depressed humoral immune function. A

dose-related increase in liver weight was observed in mice exposed to all grades of pentachlorophenol. A dose-related increase in spleen weight was observed in male mice exposed to all grades of pentachlorophenol; a decrease in spleen weight was observed in female mice exposed to all grades of pentachlorophenol except pure.

After 6 months' exposure, histopathologic examination consistently revealed effects in the liver and urinary bladder. The liver lesions were present at all doses with all four grades of pentachlorophenol but were less severe at comparable doses in the mice exposed to pure pentachlorophenol; they consisted of hepatocellular karyomegaly, cytomegaly, and degeneration. The changes in the urinary bladder consisted of a brown granular pigment in the cells of the surface epithelium. No inflammation or proliferative response was associated with the pigment.

Based primarily on the liver lesions observed in the 6-month studies, diets chosen for the 2-year studies contained 0, 100, or 200 ppm technical-grade pentachlorophenol or 0, 100, 200, or 600 ppm EC-7, fed to groups of 50 male and 50 female mice. DP-2 and pure pentachlorophenol were not chosen for the 2-year studies because of economic considerations and because the clinicopathologic syndrome observed in the 6-month studies was similar to that observed with EC-7.

Body Weights and Survival in the Two-Year Studies: Mean body weights of mice exposed to technical-grade pentachlorophenol and EC-7 were comparable to those of controls until weeks 36-82. Thereafter, a 4%-22% dose-related decrease was observed in the mid and high dose mice exposed to EC-7 and in high dose mice exposed to technical-grade pentachlorophenol. Females were more affected than males. Feed consumption by exposed mice was similar to that by controls. The average daily doses of technical-grade pentachlorophenol were approximately 17-18 or 35 mg/kg compared with 17-18, 34-37, or 114-118 mg/kg of EC-7. Survival of mice did not appear to be affected by exposure to either technical-grade pentachlorophenol or EC-7 at the doses used in these studies.

Neoplastic and Nonneoplastic Effects in the Two-Year Studies: The incidences of hepatocellular adenomas and carcinomas were increased (dose related) in male and female mice exposed to either technical-grade pentachlorophenol or EC-7, although the increase was less marked in females exposed to technical-grade pentachlorophenol (adenomas or carcinomas, combined: technical-grade: male – control, 7/32, 22%; low dose, 26/47, 55%; high dose, 37/48, 77%; female – 3/33, 9%; 9/49, 18%; 9/50, 18%; EC-7: male – control, 6/35, 17%; low dose, 19/48, 40%; mid dose, 21/48, 44%; high dose, 34/49, 69%; female – 1/34, 3%; 4/50, 8%; 6/49, 12%; 31/48, 65%).

The incidences of pheochromocytomas in male mice were significantly greater than those in controls for both technical-grade pentachlorophenol (0/31; 10/45, 22%; 23/45, 51%) and EC-7 (1/34, 3%; 4/48, 8%; 21/48, 44%; 45/49, 92%). These neoplasms were also increased in female mice exposed to EC-7 at the highest dose (0/35; 2/49, 4%; 2/46, 4%; 38/49, 78%) but not in those exposed to technical-grade pentachlorophenol (2/33, 6%; 2/48, 4%; 1/49, 2%). Hyperplasia of the adrenal medulla was observed at increased incidences in mice that received either technical-grade pentachlorophenol (male: 1/31; 10/45; female: 0/33; 4/48; 2/49) or EC-7 (male: 1/34; 19/48; 13/48; 1/49; female: 2/35; 1/49; 5/46; 17/49).

The incidences of hemangiosarcomas in the spleen and/or liver were significantly greater than those in controls for high dose female mice that received technical-grade pentachlorophenol (0/35; 3/50, 6%; 6/50, 12%) or EC-7 (0/35; 1/50, 2%; 3/50, 6%; 8/49, 16%).

Compound-related nonneoplastic lesions occurred in the liver, spleen, and nose in mice exposed to either technical-grade pentachlorophenol or EC-7. The lesions in the liver included dose-related increased incidences of clear cell foci, chronic active inflammation, pigmentation, necrosis, cytomegaly, proliferation of hematopoietic cells, and bile duct hyperplasia. Increased amounts of extramedullary hematopoiesis of the splenic red pulp were observed at increased incidences in dosed male and high dose female mice that received technical-grade pentachlorophenol (male: 5/30; 15/23; 18/46; female: 2/33; 4/13; 11/47). Acute focal inflammation of the nasal mucosa and focal metaplasia of the olfactory epithelium were observed at increased incidences in high dose mice that received EC-7 (inflammation – male: 4/35; 1/13; 3/16; 47/49; female: 0/35; 0/14; 2/5; 46/48; focal metaplasia – male: 2/35; 1/13; 2/16; 46/49; female: 1/35; 0/14; 2/5; 45/48) but not in mice exposed to technical-grade pentachlorophenol.

Genetic Toxicology: Pentachlorophenol (91.6% pure; equivalent in purity to the technical-grade pentachlorophenol used in the toxicology studies) was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in the presence or absence of exogenous metabolic activation (S9). In cytogenetic studies with cultured CHO cells, pentachlorophenol produced an increase in chromosomal aberrations in the presence but not the absence of S9 metabolic activation; conversely, sister chromatid exchanges (SCEs) were induced only in the absence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of pentachlorophenol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing technical-grade pentachlorophenol, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms. There was *some evidence of carcinogenic activity* for female B6C3F$_1$ mice exposed to technical-grade pentachlorophenol, as shown by increased incidences of hemangiosarcomas and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* for male B6C3F$_1$ mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neo-

plasms. There was *clear evidence of carcinogenic activity* for female B6C3F$_1$ mice exposed to pentachlorophenol, EC-7, as shown by increased incidences of adrenal medullary and hepatocellular neoplasms and hemangiosarcomas.

Chemically related increased incidences of nonneoplastic lesions in mice of each sex included hepatocellular cytomegaly, necrosis, inflammation, pigmentation, and clear cell foci and intrahepatic bile duct hyperplasia.

Synonyms or Common Names: chlorophen; PCP; penchlorol; penta; pentachlorofenol; pentachlorofenolo; pentachlorphenol; 2,3,4,5,6-pentachlorophenol

Trade Names: Acutox; Chem-Penta; Chem-Tol; Cryptogil ol; Dowicide 7; Dowicide EC-7; Dow Pentachlorophenol DP-2 Antimicrobial; Durotox; EP 30; Fungifen; Fungol; Glazd Penta; Grundier Arbezol; Lauxtol; Lauxtol A; Liroprem; Moosuran; Pentacon; Penta-Kil; Pentasol; Penwar; Peratox; Permacide; Permagard; Permasan; Permatox; Priltox; Permite; Santophen; Santophen 20; Sinituho; Term-i-Trol; Thompson's Wood Fix; Weedone; Witophen P

Report Date: March 1989

TR-350 Toxicology and Carcinogenesis Studies of Tribromomethane (Bromoform) (CAS No. 75-25-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Tribromomethane, a chemical intermediate and solvent, has been identified as a drinking water contaminant resulting from water chlorination. Toxicology and carcinogenesis studies were conducted by administering tribromomethane (95%-97% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once or for 14 days, 13 weeks, or 2 years.

Single-Administration, Fourteen-Day, and Thirteen-Week Studies: All rats that received 2,000 mg/kg and 3/5 males and 3/5 females that received 1,000 mg/kg tribromomethane died before the end of the single-administration studies. All mice that received 2,000 mg/kg, 4/5 males and 2/5 females that received 1,000 mg/kg, and 1/5 males that received 500 mg/kg died before the end of the studies. Shallow breathing was observed for rats and male mice that received 1,000 or 2,000 mg/kg tribromomethane.

In the 14-day studies, all rats that received 600 or 800 mg/kg and 1/5 males that received 400 mg/kg tribromomethane died before the end of the studies. The final mean body weight of male rats that received 400 mg/kg was 14% lower than that of vehicle controls. One of five male mice that received 600 mg/kg and 1/5 female mice that received 800 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control mice were comparable.

None of the rats died before the end of the 13-week studies (doses ranged from 12 to 200 mg/kg). Final mean body weights were comparable for dosed and vehicle control rats. All male rats that received 100 or 200 mg/kg tribromomethane and all female rats that received 200 mg/kg were lethargic. The incidences of cytoplasmic vacuolization of hepatocytes in dosed male rats were slightly increased compared with that in vehicle controls. The severity of this lesion was increased in the 200 mg/kg group. One of 10 female mice that received 100 mg/kg tribromomethane died before the end of the 13-week studies. The final mean body weight of mice that received 400 mg/kg was 8% lower than that of vehicle controls for males and was comparable to that of vehicle controls for females. Cytoplasmic vacuolization of hepatocytes was observed in the liver of 5/10 male mice that received 200 mg/kg and in 8/10 male mice that received 400 mg/kg tribromomethane.

Based on these results, 2-year studies of tribromomethane were conducted by administering 0, 100, or 200 mg/kg tribromomethane in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex and 50 female B6C3F$_1$ mice. Male B6C3F$_1$ mice were administered 0, 50, or 100 mg/kg tribromomethane on the same schedule.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose male and female rats were 10%-28% lower than those of vehicle controls throughout the second year of the studies. Survival of the high dose group of male rats was significantly lower than that of the vehicle controls after week 91; no significant differences in survival were observed between any groups of female rats (male: vehicle control, 34/50; low dose, 30/50; high dose, 11/50; female: 34/50; 28/50; 28/50). Reduced survival for male rats given 200 mg/kg tribromomethane lowered the sensitivity of this group to detect a carcinogenic response. Mean body weights of dosed and vehicle control male mice were comparable throughout the study. Mean body weights of dosed female mice were 5%-16% lower than those of vehicle controls from week 28 to the end of the study. No significant differences in survival were observed between any groups of male mice; the survival of both dosed groups of female mice was significantly lower than that of the vehicle controls after week 77 (male: 41/50; 37/50; 36/50; female: 25/49; 15/50; 20/50). Reduced survival in all groups of female mice was partly due to a utero-ovarian infection; nonetheless, survival of all groups of female mice was at least 50% by week 92.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Uncommon adenomatous polyps or adenocarcinomas (combined) of the large intestine (colon or rectum) were induced in three male rats (vehicle control, 0/50; low dose, 0/50; high dose, 3/50) and in nine female rats (0/50; 1/50; 8/50); the historical incidence of neoplasms of the large intestine is less than 0.2% in approximately 2,000 corn oil vehicle control male F344/N rats, and none has been observed in approximately 2,000 corn oil vehicle control female F344/N rats. Three of the neoplasms of the large intestine (one in the high dose male rats and two in the high dose female rats) were adenocarcinomas.

Focal or diffuse fatty change of the liver was observed at increased incidences in dosed rats (male: 23/50; 49/50; 50/50; female: 19/50; 39/49; 46/50). Active chronic inflammation was observed at increased incidences in dosed male and high dose female rats (male: 0/50; 29/50; 23/50; female: 9/50; 8/49; 27/50). The incidence of necrosis of the liver was increased in high dose male rats (7/50; 3/50; 20/50) and decreased in dosed females (11/50; 3/49; 2/50).

Mixed cell focus was observed at increased incidences in dosed female rats (8/50; 25/49; 28/50).

Other nonneoplastic lesions observed at increased incidences in dosed rats included chronic active inflammation and squamous metaplasia of the ducts of the salivary gland (squamous metaplasia—male: 0/50; 15/50; 31/48; female: 0/49; 10/49; 16/50; chronic active inflammation—male: 0/50; 16/50; 25/48; female: 0/49; 9/49; 18/50), squamous metaplasia of the prostate gland (2/49; 6/46; 12/50), ulcers of the forestomach (male: 1/49; 5/50; 10/50), and chronic active inflammation of the lung (male: 1/50; 7/50; 15/50). Pigmentation of the spleen was also observed at an increased incidence in high dose female rats. The salivary gland and lung lesions were characteristic of infection by rat coronavirus, a virus to which a positive serologic reaction was observed early in the studies.

The incidence of follicular cell hyperplasia of the thyroid gland was increased in high dose female mice (5/49; 4/49; 19/47), and fatty change of the liver was increased in both dosed groups of female mice (1/49; 9/50; 24/50). No chemically related adverse effects were observed in male mice.

Neoplastic lesions that occurred at lower incidences in dosed animals compared with those in vehicle controls included preputial gland neoplasms in male rats (10/41; 5/38; 1/34), uterine stromal polyps in female rats (10/49; 9/50; 2/50), anterior pituitary gland adenomas in male and female rats (male: 12/50; 12/48; 2/45; female: 29/48; 12/46; 16/48), mammary gland fibroadenomas in female rats (22/50; 17/50; 6/50), and alveolar/bronchiolar neoplasms in male mice (11/50; 7/50; 2/49). Other than concomitant decreases in body weights, no other reasons are obvious to correlate these decreases with chemical administration.

Genetic Toxicology: Tribromomethane exhibited equivocal mutagenicity in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation and in strains TA97 and TA98 when exposure occurred in the presence of hamster S9; tribromomethane produced no increases in revertant colonies in TA1535 or TA1537 with or without exogenous metabolic activation. Tribromomethane induced trifluorothymidine (Tft) resistance in mouse L5178Y cells with and without metabolic activation. When tested in cultured Chinese hamster ovary (CHO) cells for cytogenetic effects, tribromomethane produced an increase in both sister chromatid exchanges (SCEs) and chromosomal aberrations in the absence, but not in the presence, of exogenous metabolic activation. Tribromomethane caused sex-linked recessive lethal mutations in Drosophila when administered to adult males by feeding; no induction of mutations was observed when tribromomethane was administered by abdominal injection. Results of tests for reciprocal translocations in adult male Drosophila exposed to tribromomethane by feeding were negative. In vivo tests for cytogenetic effects in bone marrow cells of male $B6C3F_1$ mice demonstrated that intraperitoneal injection of tribromomethane induced an increase in SCEs but no increase in chromosomal aberrations. Intraperitoneal injection of tribromomethane also induced an increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of $B6C3F_1$ mice.

Audit: The data, documents, and pathology materials from the 2-year studies of tribromomethane have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of tribromomethane for male F344/N rats and *clear evidence of carcinogenic activity* for female F344/N rats, based on increased incidences of uncommon neoplasms of the large intestine. Reduced survival for male rats given 200 mg/kg tribromomethane lowered the sensitivity of this group to detect a carcinogenic response. Chemically related nonneoplastic lesions included fatty change and active chronic inflammation of the liver in male and female rats, minimal necrosis of the liver in male rats, and mixed cell foci of the liver in female rats. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice given 50 or 100 mg/kg tribromomethane or for female $B6C3F_1$ mice given 100 or 200 mg/kg; male mice might have been able to tolerate a higher dose. Survival of female mice was reduced, partly due to a utero-ovarian infection.

Synonym: bromoform

Report Date: May 1989

TR-351 Toxicology and Carcinogenesis Studies of *para*-Chloroaniline Hydrochloride (CAS No. 20265-96-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

p-Chloroaniline has a large production volume and is used as a dye intermediate. Toxicology and carcinogenesis studies of *p*-chloroaniline (greater than 99% pure) were conducted by administering *p*-chloroaniline hydrochloride in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Vehicle controls were given deionized water by gavage. All doses were calculated as *p*-chloroaniline; the chemical was administered as the hydrochloride after dissolution in water containing molar equivalents of hydrochloric acid. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells. Hematologic parameters were measured at the end of the

13-week studies and at 6, 12, 18, and 24 months in the 2-year studies. Supplemental studies of the distribution and disposition of *p*-chloroaniline were conducted in male F344 rats.

Sixteen-Day and Thirteen-Week Studies: In the 16-day studies, male and female rats and mice received 25, 50, 100, or 400 mg/kg of body weight. The vehicle controls received deionized water. All rats and mice that received 200 or 400 mg/kg died during the first 6 days of the studies. Some deaths occurred in each of the lower dose groups of mice. Splenic enlargement was observed at necropsy in rats administered 25, 50, or 100 mg/kg. Congestion of the spleen and hemosiderin deposition in the renal cortical tubular epithelial cells were observed at 100 mg/kg in male and female rats. Compound-related lesions in mice included hemosiderosis of the liver Kupffer cells and congestion of the spleen.

In the 13-week studies, 10 rats of each sex were administered doses of 0, 5, 10, 20, 40, or 80 mg/kg. All male rats lived to the end of the 13-week studies. One of 10 female rats that received 80 mg/kg died from unknown causes. The final mean body weights of rats that received 80 mg/kg were 16% lower than that of vehicle controls for males and 4% lower for females. In the 13-week studies in mice, 10 animals of each sex were administered doses of 0, 7.5, 15, 30, 60, or 120 mg/kg. Deaths in mice were not related to *p*-chloroaniline hydrochloride administration. The final mean body weights of dosed and vehicle control mice were similar. In both rats and mice, no chemically related effects on organ weights were observed at necropsy, except for the spleen, which was enlarged as a function of increasing dose. Methemoglobin was increased in dosed groups and resulted in a secondary anemia, the severity of which was dose related. Compound-related lesions observed histologically, including pigmentation (hemosiderin) in the kidney, spleen, and liver and hematopoiesis in the liver and spleen, reflected the response to the hemolytic anemia and methemoglobinemia induced by *p*-chloroaniline hydrochloride.

Based on these results, groups of 50 rats of each sex were administered 2, 6, or 18 mg/kg *p*-chloroaniline hydrochloride in water by gavage, 5 days per week for 103 weeks. Groups of 50 mice of each sex were administered 3, 10, or 30 mg/kg on the same schedule.

Metabolism and Disposition Studies in Rats: The metabolism and disposition studies in F344/N rats showed that metabolic and excretory pathways were not saturated by *p*-chloroaniline administered orally at doses ranging from 0.3 to 30 mg/kg. *p*-Chloroaniline was rapidly metabolized and excreted primarily in urine with a half-life of approximately 2 hours.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were generally within 5% of those of vehicle controls throughout the studies. The survival of the low and mid dose groups of male rats and of the low and high dose groups of female rats was significantly greater than that of the vehicle controls (male: vehicle control, 18/49; low dose, 32/50; mid dose, 32/50; high dose, 21/50; female: 27/50; 39/50; 36/50; 37/50). The increased survival was attributed to the decreased incidences of mononuclear cell leukemia. Mean body weights of high dose male and female mice were generally within 5% of those of vehicle controls throughout the studies. The survival of the mid dose group of male mice was lower than that of the vehicle controls after week 99 (male: 43/50; 36/50; 29/50; 35/50; female: 39/50; 42/50; 44/50; 41/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Fibrosis of the spleen was increased in dosed male and high dose female rats (male: vehicle control, 3/49; low dose, 11/50; mid dose, 12/50; high dose, 41/50; female: 1/50; 2/50; 3/50; 42/50). Cellular infiltration of lipocytes (fatty metaplasia) was observed in the spleen at increased incidences in high dose rats (male: 0/49; 0/50; 0/50; 24/50; female: 0/50; 0/50; 0/50; 11/50). The incidence of uncommon sarcomas of the spleen in high dose male rats was significantly greater than that in the vehicle controls (fibrosarcomas, osteosarcomas, or hemangiosarcomas, combined: 0/49; 1/50; 3/50; 38/50). Many of these tumors metastasized to one or more sites. In female rats, one fibrosarcoma of the spleen was found in a mid dose animal, and one osteosarcoma of the spleen was found in a high dose animal. The historical incidence of splenic connective tissue sarcomas (all types) in water gavage vehicle controls is 1/298 (0.3%) for male rats and 0/297 for female rats. The historical incidence of hemangiosarcomas in water gavage controls is 0/300 for male rats and 1/297 (0.3%) for female rats.

Adrenal medullary hyperplasia was observed at an increased incidence in high dose female rats (4/50; 4/50; 7/50; 24/50). Marginally increased incidences of pheochromocytomas were seen in high dose male (13/49; 14/48; 15/48; 26/49) and female (2/50; 3/50; 1/50; 6/50) rats. The historical incidence of pheochromocytomas in water gavage vehicle control male F344/N rats is 121/299 (40% ± 16%); the historical incidence in water gavage vehicle control female F344/N rats is 20/295 (7% ± 2%).

The incidences of mononuclear cell leukemia in dosed male and female rats were lower than those in vehicle controls (male: 21/49; 3/50; 2/50; 3/50; female: 10/50; 2/50; 1/50; 1/50). The incidences of malignant lymphomas in dosed male and female mice were lower than those in vehicle controls (male: 10/50; 3/49; 9/50; 3/50; female: 19/50; 12/50; 5/50; 10/50).

Hematologic and methemoglobin measurements were made on blood samples collected from 15 randomly selected male and female rats per dose group at 6, 12, 18, and 24 months. In general, the high dose group at various intervals showed mild hemolytic anemia and dose-related increases in methemoglobin.

In rats, compound-related nonneoplastic lesions were seen histopathologically in the bone marrow, spleen, and liver. These lesions included bone marrow hyperplasia, hepatic hemosiderosis, and splenic fibrosis and suggest compound-related effects on the hematopoietic system in general, the erythropoietic system specifically, and mesenchymal cells in the spleen.

In male mice, the incidence of hemangiosarcomas of the liver or spleen in high dose male mice was greater than that

in the vehicle controls (4/50; 4/49; 1/50; 10/50). The historical incidence of hemangiomas or hemangiosarcomas at all sites (combined) in water gavage vehicle control male $B6C3F_1$ mice is 11/350 (3% ± 3%).

The incidences of hepatocellular adenomas or carcinomas (combined) were increased in dosed male mice (11/50; 21/49; 20/50; 21/50), primarily due to increased incidences of hepatocellular carcinomas (3/50; 7/49; 11/50; 17/50). Hepatocellular carcinomas metastasized to the lung in 1/50 vehicle control, 1/49 low dose, 2/50 mid dose, and 9/50 high dose male mice. The historical incidence of hepatocellular neoplasms in water gavage vehicle controls is 106/347 (31 ± 6%).

Genetic Toxicology: *p*-Chloroaniline was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of exogenous metabolic activation; no increase in revertant colonies was observed in strains TA97, TA1535, or TA1537. *p*-Chloroaniline induced trifluorothymidine (Tft) resistance in mouse L5178Y lymphoma cells with and without metabolic activation. In cultured CHO cells, treatment with *p*-chloroaniline produced significant increases in sister chromatid exchanges (SCEs) both with and without metabolic activation (S9); chromosomal aberrations were significantly increased only in the presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of *p*-chloroaniline have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year water gavage studies, there was *clear evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for male F344/N rats, as indicated by increased incidences of uncommon sarcomas of the spleen. Pheochromocytomas of the adrenal gland may also have been associated with chemical administration. There was *equivocal evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female F344/N rats, as indicated by the presence of uncommon sarcomas of the spleen in one mid and one high dose animal and the increased incidence of pheochromocytomas of the adrenal gland. There was *some evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for male $B6C3F_1$ mice, as indicated by increased incidences of hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen. There was *no evidence of carcinogenic activity* of *p*-chloroaniline hydrochloride for female $B6C3F_1$ mice administered 3, 10, or 30 mg/kg by gavage for 2 years.

The incidences of mononuclear cell leukemia in male and female rats and of malignant lymphomas in male and female mice were decreased by administration of *p*-chloroaniline hydrochloride. Compound-related splenic fibrosis was present in male and female rats.

Synonyms: 1-amino-4-chlorobenzene hydrochloride; 4-chlorophenylamine hydrochloride; 4-chlorobenzeneamine hydrochloride

Report Date: July 1989

TR-352 Toxicology and Carcinogenesis Studies of *N*-Methylolacrylamide (CAS No. 924-42-5) in F344/N Rats and $B6C3F_1$ Mice

N-Methylolacrylamide is a cross-linking agent used in adhesives, binders for paper, crease-resistant textiles, resins, latex film, and sizing agents. Toxicology and carcinogenesis studies were conducted by administering *N*-methylolacrylamide (98% pure) in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. In vitro genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells; an in vivo bone marrow micronucleus test was performed with $B6C3F_1$ mice. Neurobehavioral assays were performed during the 13-week studies.

Sixteen-Day Studies: The doses of *N*-methylolacrylamide used ranged from 25 to 400 mg/kg. All rats that received 400 mg/kg died within 4 days, and 3/5 male rats that received 200 mg/kg also died before the end of the studies. Compound-related clinical signs seen with 200 mg/kg included ataxia, muscle tremors, and hyperirritability. Ataxia after dosing was observed from day 7 to the end of the studies for rats that received 100 mg/kg. The final mean body weight of male rats that received 100 or 200 mg/kg was 10% or 27% lower than that of the vehicle controls. The final mean body weight of female rats that received 200 mg/kg was 20% lower than that of the vehicle controls. Compound-related lesions in rats included hyperplasia of the bronchiolar and tracheal epithelium, dysplasia of the nasal and tracheal epithelium, centrilobular hepatocellular necrosis, lymphoid depletion of the spleen, and myelin degeneration of the lumbar ventral spinal nerve.

All 5 male and 4/5 female mice that received 400 mg/kg *N*-methylolacrylamide died on the second day of the 16-day studies. The surviving female mouse in the 400 mg/kg group and the male and female mice in the 200 mg/kg groups were ataxic after they were dosed, starting on day 2. Weight changes were inconsistent among dose groups. Bronchial epithelial hyperplasia (mild) appeared to be dose related in males and females. Sinusoidal congestion of the liver and vacuolar degeneration of myocardial fibers were seen in males and females given 400 mg/kg.

Thirteen-Week Studies: The doses of *N*-methylolacrylamide used ranged from 12.5 to 200 mg/kg. All rats that received 100 or 200 mg/kg died before the end of the studies. Rats that received 100 or 200 mg/kg had hind limb ataxia, which progressed to hind limb paralysis. Rats that received 50 mg/kg had hind limb ataxia beginning at week 8, which progressed to hind limb paresis by week 11. The final mean body weight of rats that received 25 or 50 mg/kg was 8% or 16% lower than that of the vehicle controls for males and 6% or 10% lower for females. In neurobehavioral assessments, decreased forelimb and hind limb grip strength was seen at doses as low as 25 mg/kg for female rats and at doses as low as 12.5 mg/kg for male rats. A decreased startle response was seen for females at doses as low as 25 mg/kg. The landing

foot spread was significantly increased for male and female rats that received 50 mg/kg.

Axon filament and myelin sheath degeneration of the brain stem, spinal cord, and/or peripheral nerves was seen in rats at increased incidences at 25 mg/kg and higher doses. Inflammation and/or hemorrhage and edema of the urinary bladder mucosa were seen with doses of 25 mg/kg or more in a few rats that had distended bladders at gross examination.

All mice that received 200 mg/kg *N*-methylolacrylamide died before the end of the studies. Final mean body weights of dosed and vehicle control mice were similar. A decreased relative testis weight was observed for mice that received 12.5 mg/kg or more. The relative kidney weights for male mice receiving 50 or 100 mg/kg were greater than that for vehicle controls. Neurobehavioral studies indicated decreased forelimb grip strength in male and female mice at doses as low as 25 mg/kg. An exaggerated startle response was seen for female mice given 100 mg/kg. A reduction in rotarod performance was seen for male and female mice receiving 100 mg/kg and for male mice receiving 25 mg/kg.

Hepatocellular necrosis and thymic lymphocytic necrosis were compound-related effects in mice given 200 mg/kg *N*-methylolacrylamide. Hemorrhage, necrosis, and mineralization of the zona reticularis of the adrenal gland were present in 3/10 female mice given 200 mg/kg, and cytoplasmic vacuolization of the adrenal cortex was seen with lower doses.

Based on the results of these short-term studies, 2-year studies were conducted by administering 0, 6, or 12 mg/kg *N*-methylolacrylamide in water by gavage, 5 days per week for 103 weeks, to groups of 50 rats of each sex. Groups of 50 mice of each sex were administered 0, 25, or 50 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were within 6% of those of vehicle controls throughout most of the studies. Mean body weights of dosed mice were as much as 25% greater than those of vehicle controls for females and as much as 13% greater for males. The survival of female rats given 25 mg/kg per day was lower than that of vehicle controls after day 550, but survival of female rats given 50 mg/kg per day was not different from that of vehicle controls (vehicle control, 35/50; low dose, 22/50; high dose, 33/50). No differences in survival were observed between any other groups of rats or mice of either sex (male rats: 28/50; 22/50; 27/50; male mice: 30/50; 20/50; 21/50; female mice: 41/50; 35/50; 33/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In rats, no biologically important nonneoplastic or neoplastic lesions were attributed to administration of *N*-methylolacrylamide. Higher doses might have increased the sensitivity of the studies to determine the presence or absence of a carcinogenic response.

In mice, the incidences of adenomas of the Harderian gland were increased in males given either dose of *N*-methylolacrylamide and in females given the top dose (male: vehicle control, 1/48; low dose, 14/49; high dose, 29/50; female: 5/47; 8/45; 20/48). The incidences of carcinomas of the Harderian gland were not significantly increased by *N*-methylolacrylamide administration (male: 1/48; 0/49; 2/50; female: 0/47; 3/45; 2/48).

The incidences of hepatocellular adenomas were increased in male and female mice given 50 mg/kg *N*-methylolacrylamide (male: 8/50; 4/50; 19/50; female: 3/50; 4/50; 17/49). The incidences of hepatocellular carcinomas were also marginally increased in dosed male mice (male: 6/50; 13/50; 12/50; female: 3/50; 3/50; 2/49). Hepatocellular adenomas and carcinomas (combined) occurred with positive trends, and the incidences in male and female mice receiving 50 mg/kg were increased compared with those in the vehicle controls (male: 12/50; 17/50; 26/50; female: 6/50; 7/50; 17/49).

Chronic inflammation and alveolar epithelial hyperplasia of the lung were observed at increased incidences in mice given *N*-methylolacrylamide. Sentinel mice were seropositive for Sendai virus at 18 months. The incidences of alveolar/bronchiolar adenomas (3/49; 6/50; 11/50) and carcinomas (2/49; 4/50; 10/50) were increased in male mice given 50 mg/kg. Alveolar/bronchiolar adenomas or carcinomas (combined) occurred with a positive trend in male mice (5/49; 10/50; 18/50). The incidence of alveolar/bronchiolar adenomas or carcinomas (combined) was increased in female mice given the top dose of 50 mg/kg (6/50; 8/50; 13/49).

Ovarian atrophy was observed at increased incidences in female mice receiving *N*-methylolacrylamide (3/50; 39/45; 38/47). The incidences of benign granulosa cell tumors were also increased in the dosed groups (0/50; 5/45; 5/47).

The incidence of adenomas of the pars distalis in high dose female mice was significantly lower than that in vehicle controls (13/49; 5/14; 4/43).

Genetic Toxicology: *N*-Methylolacrylamide was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 when tested with or without exogenous metabolic activation. *N*-Methylolacrylamide induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells with and without metabolic activation. No increase in micronucleated polychromatic erythrocytes (PCEs) was observed in the bone marrow of $B6C3F_1$ mice after intraperitoneal injection of *N*-methylolacrylamide.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of *N*-methylolacrylamide for male or female F344/N rats receiving doses of 6 or 12 mg/kg per day by aqueous gavage. There was *clear evidence of carcinogenic activity* of *N*-methylolacrylamide for male $B6C3F_1$ mice, based on increased incidences of neoplasms of the Harderian gland, liver, and lung. There was *clear evidence of carcinogenic activity* of *N*-methylolacrylamide for female $B6C3F_1$ mice, based on increased incidences of neoplasms of the Harderian gland, liver, lung, and ovary.

In rats, because no biologically important toxic effects were attributed to *N*-methylolacrylamide administration, somewhat higher doses could have been used to increase the sensitivity of these studies for determining the pres-

ence or absence of a carcinogenic response. In female mice, ovarian atrophy was compound related.

Synonyms: *N*-(hydroxymethyl)acrylamide; *N*-(hydroxymethyl)-2-propenamide; *N*-methanolacrylamide; monomethylolacrylamide

Report Date: September 1989

TR-353 Toxicology and Carcinogenesis Studies of 2,4-Dichlorophenol (CAS No. 120-83-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

2,4-Dichlorophenol is a chemical intermediate used principally in the manufacture of the herbicide 2,4-dichlorophenoxyacetic acid. Toxicology and carcinogenesis studies were conducted by feeding diets containing 2,4-dichlorophenol (greater than 99% pure) for 14 days, 13 weeks, or 2 years to groups of F344/N rats and B6C3F$_1$ mice of each sex. Genetic toxicology tests were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, male and female rats and mice were given diets containing 2,4-dichlorophenol at concentrations up to 40,000 ppm. One high dose male mouse died before the end of the studies; no deaths occurred in any other group, and no compound-related lesions were seen at necropsy in rats or mice. In the 13-week studies, groups of 10 rats and 10 mice of each sex were fed diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm 2,4-dichlorophenol. All rats lived to the end of the studies, whereas all mice that received 40,000 ppm died during the first 3 weeks of the studies. Final mean body weights of rats that received 20,000 or 40,000 ppm and of male mice that received 20,000 ppm were at least 10% lower than those of controls. Bone marrow atrophy in rats and necrosis and syncytial alteration (multinucleated hepatocytes) in the liver of male mice were compound-related effects. Two-year studies were conducted by feeding diets containing 0, 5,000, or 10,000 ppm 2,4-dichlorophenol to groups of 50 male rats and 50 male and 50 female mice for 103 weeks. Groups of 50 female rats received diets containing 0, 2,500, or 5,000 ppm.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male and female rats, high dose male mice, and both dosed groups of female mice were generally lower than those of controls. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: control, 33/50; low dose, 25/50; high dose, 32/50; female rats: 34/50; 43/50; 40/50; male mice: 33/50; 32/50; 31/50; female mice: 45/50; 40/50; 43/50). The average daily feed consumption by rats in the low dose and high dose groups was 94%-97% that by the controls. The estimated daily mean consumption of 2,4-dichlorophenol was 210 or 440 mg/kg for low dose or high dose male rats and 120 or 250 mg/kg for low dose or high dose female rats. The average daily feed consumption by mice in the low dose and high dose groups was 97% and 78% of that by the controls for males and 94% and 85% for females. The estimated daily mean consumption of 2,4-dichlorophenol was 800 or 1,300 mg/kg for low dose or high dose male mice and 430 or 820 mg/kg for low dose or high dose female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: There were no compound-related increased incidences of neoplastic lesions in rats or mice. The incidence of mononuclear cell leukemia was decreased in dosed male rats relative to that in controls (control, 31/50; low dose, 17/50; high dose, 17/50); the incidence of malignant lymphomas was decreased in high dose female mice (4/50) relative to that in controls (12/50). Syncytial alteration of hepatocytes was observed at increased incidences in dosed male mice (11/50; 33/49; 42/48).

Genetic Toxicology: The mutagenic effect of 2,4-dichlorophenol in *S. typhimurium* strain TA1535 was considered to be equivocal only in the presence of hamster S9; 2,4-dichlorophenol produced no increases in revertant colonies in strains TA98, TA100, or TA1537 with or without exogenous metabolic activation. 2,4-Dichlorophenol increased trifluorothymidine (Tft) resistance in the mouse L5178Y assay without metabolic activation; it was not tested with activation. In cultured CHO cells, 2,4-dichlorophenol did not induce chromosomal aberrations but did significantly increase the frequency of sister chromatid exchanges (SCEs) both in the presence and absence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of 2,4-dichlorophenol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* for male F344/N rats fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol or for female F344/N rats fed diets containing 2,500 or 5,000 ppm 2,4-dichlorophenol. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol.

Synonyms: 2,4-DCP; 2,4-dichlorohydroxybenzene

Report Date: June 1989

TR-354 Toxicology and Carcinogenesis Studies of Dimethoxane (CAS No. 828-00-2) (Commercial Grade) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Dimethoxane is used as an antimicrobial agent in water-based paints, dyestuffs, fabric softeners, sizings, and spinning emulsions. In the past, it was used in lipsticks and other cosmetic preparations. Toxicology and carcinogenesis studies were conducted by administering

commercial-grade dimethoxane (80% pure, none of these impurities exceeded 3%) in corn oil gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex one time or 5 days per week for 16 days, 13 weeks, 15 months, or 2 years. Clinical pathology analyses were performed at 15 months in the 2-year studies. Commercial-grade dimethoxane was studied because that is the grade to which humans are generally exposed. The same lot of commercial-grade dimethoxane was used in genetic toxicology tests for mutagenicity in *Salmonella typhimurium*, for sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells, and for sex-linked recessive lethal mutations and translocation in Drosophila.

Sixteen-Day Studies: In the 16-day studies, rats and mice received 0, 125, 250, 500, 1,000, or 2,000 mg/kg dimethoxane in corn oil per day. Deaths occurred in rats and in male mice that received 2,000 mg/kg. Body weights of rats and mice were similar to those of vehicle controls. Compound-related clinical signs were not seen in surviving rats. Hemorrhage and necrosis of the stomach were observed in rats in the 2,000 mg/kg group which died before the end of the studies. Lesions of the forestomach, including inflammation, hyperplasia, hyperkeratosis, and ulceration, occurred in rats that received 250-2,000 mg/kg. Mice that received 500-2,000 mg/kg dimethoxane had lesions of the forestomach including erosion, ulceration, hyperplasia, and hyperkeratosis. Forestomach lesions were not seen at 125 or 250 mg/kg.

Thirteen-Week Studies: No compound-related deaths occurred in rats. Doses used were 0, 31, 62, 125, 250, or 500 mg/kg dimethoxane in corn oil by gavage. The final mean body weights of rats that received 500 mg/kg were 17% lower than that of vehicle controls for males and 5% lower for females. Ulceration, inflammation, and acanthosis with hyperkeratosis of the stratified squamous epithelium of the forestomach were seen in rats that received 500 mg/kg. Forestomach lesions were not seen in males that received 31 mg/kg or in females that received 31, 62, or 125 mg/kg.

All mice lived to the end of the studies (doses used were 0, 31, 62, 125, 250, or 500 mg/kg dimethoxane in corn oil by gavage). Final mean body weights of dosed and vehicle control mice were similar. Minimal-to-mild acanthosis and hyperkeratosis of the squamous epithelium of the forestomach were seen in 4/10 high dose male and 1/10 high dose female mice.

Because of the forestomach lesions observed in rats and mice and reduced body weight observed for male rats, doses selected for the 2-year studies were 0, 62.5, or 125 mg/kg dimethoxane in corn oil, given by gavage 5 days per week to groups of 60 male rats; 0, 125, or 250 mg/kg to groups of 60 female rats; and 0, 250, or 500 mg/kg to groups of 58 or 60 mice of each sex. Ten animals per sex and species from each dose group were killed 15 months after initiation of the studies to determine toxicity, preneoplastic lesions, and early induced neoplasia.

Fifteen-Month Studies: Minimal diffuse acanthosis and hyperplasia of the forestomach were seen in 7/10 female rats at 250 mg/kg, 7/10 males at 125 mg/kg, and 1/9 male and 1/9 female vehicle controls. Acanthosis of the forestomach was seen in 7/10 male and 6/10 female mice at 500 mg/kg. Harderian gland adenomas were seen in one high dose male and one high dose female mouse. A harderian gland adenocarcinoma was seen in a second high dose female mouse. No compound-related effects were observed for clinical chemical or hematologic values or for organ weights for rats or mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and vehicle control rats and mice of each sex were generally similar. No significant differences in survival were observed between any groups of rats (male: vehicle control, 23/50; low dose, 28/50; high dose, 21/50; female: 30/50; 31/50; 24/50) or mice (male: 33/50; 27/48; 29/50; female: 36/50; 35/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: At no site was a significantly increased incidence of neoplastic lesions observed in dosed male or female rats or in dosed female mice. Acanthosis and hyperkeratosis were increased in the forestomach of high dose rats; acanthosis, hyperkeratosis, focal hyperplasia, and chronic active inflammation were increased in the forestomach of dosed mice. The incidence of squamous cell papillomas of the forestomach was increased in high dose male mice (vehicle control, 2/47; low dose, 3/47; high dose, 7/50). A squamous cell carcinoma of the forestomach was present in another high dose male mouse. Although the incidence of squamous cell papillomas in the high dose group was not significantly different from that in the vehicle controls, the incidence exceeded the highest observed in historical corn oil gavage vehicle controls (3/49). Other than a single squamous cell papilloma in the esophagus of a low dose male mouse, no hyperplastic or neoplastic lesions were seen outside the stomach of dosed mice which could be related to the administration of dimethoxane. Despite the observation of three harderian gland neoplasms in mice killed at 15 months, no increase in the incidences of harderian gland neoplasms was seen in dosed mice in the 2-year studies (male: 2/48; 2/48; 2/48; female: 2/48; 0/49; 2/50).

Genetic Toxicity: Dimethoxane was mutagenic in strain TA100 of *S. typhimurium* in the presence but not the absence of exogenous metabolic activation; it was not mutagenic in strains TA98, TA1535, or TA1537 with or without activation. Dimethoxane induced SCEs and chromosomal aberrations in CHO cells both with or without exogenous metabolic activation. Dimethoxane induced sex-linked recessive lethal mutations in Drosophila when administered by abdominal injection to adult males; no induction of reciprocal translocations was observed in adult males after injection of dimethoxane.Conclusions: Under the conditions of these 2-year corn oil gavage studies, there was *no evidence of carcinogenic activity* of dimethoxane for male F344/N rats receiving 62.5 or 125 mg/kg or for female F344/N rats receiving 125 or 250 mg/kg per day. There was *equivocal evidence of carcinogenic activity* of dimethoxane for male B6C3F$_1$ mice, as indicated by an increased incidence of fore-

stomach neoplasms. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice receiving 250 or 500 mg/kg per day. Acanthosis and hyperkeratosis occurred at increased incidences in the forestomach of high dose rats. Inflammation, acanthosis with hyperkeratosis, and focal hyperplasia occurred at increased incidences in the forestomach of dosed mice.

Synonyms: acetomethoxan; acetomethoxane; 6-acetoxy-2,4-dimethyl-*m*-dioxane; 2,6-dimethyl-*m*-dioxan-4-yl acetate; 2,6-dimethyl-*m*-dioxan-4-ol acetate; 2,6-dimethyl-1,3-dioxan-4-ol acetate

Report Date: September 1989

TR-355 Toxicology and Carcinogenesis Studies of Diphenhydramine Hydrochloride (CAS No. 147-24-0) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Diphenhydramine hydrochloride is a widely used antihistaminic drug in human and veterinary medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing USP-grade diphenhydramine hydrochloride (greater than 99% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, dietary concentrations ranged from 620 to 10,000 ppm for rats and from 310 to 5,000 ppm for mice. All rats that received diets containing 10,000 ppm and 9/10 rats that received diets containing 5,000 ppm died before the end of the studies. The final mean body weights of rats receiving 1,250 or 2,500 ppm were 12%-13% or 30%-34% lower than those of controls. Feed consumption by rats at the three highest concentrations was more than 30% less than that by controls. All mice receiving 5,000 ppm, 4/5 males and 4/5 females receiving 2,500 ppm, and 4/5 males receiving 1,250 ppm died before the end of the studies. The final mean body weights of mice that received 1,250 or 2,500 ppm were lower than the initial weights. All dosed rats and mice were hyperactive and sensitive to sound and/or touch.

In the 13-week studies, dietary concentrations of diphenhydramine hydrochloride ranged from 156 to 2,500 ppm for rats and from 78 to 1,250 ppm for mice. All rats lived to the end of the studies. The final mean body weights of rats receiving 1,250 or 2,500 ppm were about 15% or 35% lower than those of controls. The final mean body weight of female rats receiving 625 ppm was 9% lower than that of controls. Increased activity was observed for all male and female rats receiving 1,250 and 2,500 ppm. Cytoplasmic vacuolization of the liver, characteristic of fat accumulation, was observed in male and female rats receiving 313-2,500 ppm. The severity of this change increased with increased dose. For mice, 1/10 males receiving 313 ppm, 2/10 males receiving 625 ppm, and 8/10 males receiving 1,250 ppm died before the end of the studies. The final mean body weights of mice that received 625 or 1,250 ppm were about 9% or 16% lower than those of controls. No compound-related histopathological effects were observed in mice.

Based on the mortality and body weight effects of diphenhydramine hydrochloride in the short-term studies, dietary concentrations selected for the 2-year studies were 0, 313, and 635 ppm diphenhydramine hydrochloride for male rats and 0, 156, and 313 ppm for female rats and male and female mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and control rats were similar throughout the studies, and mean body weights of dosed mice were 3%-13% lower than those of controls throughout most of the studies. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: control, 29/50; low dose, 32/50; high dose, 24/50; female rats: 35/50; 32/50; 36/50; male mice: 29/50; 30/50; 24/48; female mice: 37/50; 39/50; 32/50). The estimated average daily feed consumption by dosed rats and dosed mice was similar to that by controls. The average amount of diphenhydramine hydrochloride consumed per day was approximately 13 or 27 mg/kg for low dose or high dose male rats, 7 or 15 mg/kg for low dose or high dose female rats, and 21 or 46-47 mg/kg for low dose or high dose male and female mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: For three high dose male rats, astrocytomas were found in brain sections taken by routine sampling procedures. Gliomas, containing neoplastic astrocytes and oligodendrocytes, were found in one control and one additional high dose male rat. The incidence of glial cell tumors in high dose male rats (4/50) exceeded the highest incidence in historical controls in the Program (2/50). The historical incidence of glial cell tumors is less than 0.7% in approximately 2,000 untreated control male F344/N rats. Three additional sections of brain were prepared from the residual fixed tissues of each male and female rat. One additional astrocytoma in a high dose male rat and one astrocytoma in a high dose female rat were observed in these sections.

Adenomas of the anterior pituitary gland in female rats occurred with a significant positive trend; the incidences in low dose male and high dose female rats were marginally greater than those in controls (male: control, 11/49; low dose, 21/50; high dose, 14/49; female: 23/50; 26/50; 35/50).

The incidence of alveolar/bronchiolar adenomas in low dose male rats was slightly greater than that in controls (0/49; 5/50; 3/50). The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in dosed male rats were not significantly different from that in controls (1/49; 6/50; 5/50) but exceeded the highest incidence in historical controls (4/49). The historical incidence of alveolar/bronchiolar neoplasms in untreated control male F344/N rats is approximately 2.2%. Adenomatous hyperplasia of the lung was not increased in incidence in dosed male rats compared with controls.

The incidences of granulomas of the liver were increased in dosed rats (male: 0/49; 3/50; 4/50; female: 8/50; 15/49; 18/50).

At no site were the incidences of neoplastic lesions in dosed mice considered to be compound related. Cytoplasmic vacuolization (fatty metamorphosis) of the liver was observed at an increased incidence in high dose female mice (0/49; 1/49; 8/49).

Genetic Toxicology: Diphenhydramine hydrochloride was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in either the presence or absence of exogenous metabolic activation. Exposure to this chemical did not induce trifluorothymidine (Tft) resistance in mouse L5178Y lymphoma cells with or without metabolic activation. In cytogenetic tests with cultured CHO cells, diphenhydramine hydrochloride induced chromosomal aberrations in the absence, but not the presence, of exogenous metabolic activation (S9); no induction of sister chromatid exchanges (SCEs) was observed in these cells with or without S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of diphenhydramine hydrochloride for male F344/N rats, based on marginally increased incidences of uncommon brain neoplasms (astrocytomas or gliomas) and of alveolar/bronchiolar neoplasms. There was *equivocal evidence of carcinogenic activity* for female F344/N rats, based on a marginal increase in the incidence of pituitary gland adenomas. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 156 or 313 ppm diphenhydramine hydrochloride.

Synonyms: 2-diphenylmethoxy-*N,N*-dimethylethanamine hydrochloride; 2-(benzhydryloxy)-*N,N*-dimethylethylamine hydrochloride; β-dimethylaminoethyl benzhydryl ether hydrochloride; benzhydramine hydrochloride

Trade Names: Alleran; Benadryl

Report Date: September 1989

TR-356 Toxicology and Carcinogenesis Studies of Furosemide (CAS No. 54-31-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Furosemide is a diuretic used in human and veterinary medicine. Toxicology and carcinogenesis studies were conducted by feeding diets containing furosemide (99% pure, USP grade) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: Dietary concentrations of furosemide used in the 14-day studies for rats and mice ranged up to 46,000 ppm. Two of five male and 3/5 female rats that received 46,000 ppm furosemide died before the end of the studies. Rats that received 15,300 or 46,000 ppm lost weight over the course of the studies. The final mean body weights of rats that received 1,700 or 5,100 ppm were 12% or 23% lower than that of controls for males and 8% or 16% lower for females. Nephrosis was dose related in rats. All five male and 1/5 female mice that received 46,000 ppm furosemide died before the end of the 14-day studies. Male mice that received 15,300 ppm and female mice that received 46,000 ppm lost weight. The final mean body weights of male mice that received 1,700 or 5,100 ppm were 16% or 14% lower than that of controls. The final mean body weight of females that received 15,300 ppm was 13% lower than that of controls. Slight dilatation of the renal cortical tubules and/or nephrosis were dose related in mice.

Dietary concentrations of furosemide used in the 13-week studies were 0 and 625-10,000 ppm for male rats and 0 and 938-15,000 ppm for female rats and male mice. Concentrations for female mice were 0 and 1,250-20,000 ppm. None of the rats died before the end of the studies. The final mean body weights of male rats that received 2,500, 5,000, or 10,000 ppm furosemide were 11%, 22%, or 44% lower than that of controls. The final mean body weights of female rats that received 3,750, 7,500, or 15,000 ppm were 18%, 26%, or 35% lower than that of controls. Minimal-to-mild nephrosis occurred in the two highest dose groups of male and female rats. Mineralization of minimal to mild severity was observed at the renal corticomedullary junction in dosed male rats receiving 625 ppm or more; the severity and incidence of the mineralization increased with increased dose. No compound-related deaths occurred in mice. The final mean body weights of male mice that received 3,750, 7,500, or 15,000 were 12%, 22%, or 17% lower than that of controls. Final mean body weights of dosed and control female mice were comparable. Compound-related lesions in mice induced minimal-to-mild nephrosis.

Because of the lower body weights and the kidney lesions in the 13-week studies, doses selected for the 2-year studies were 0, 350, or 700 ppm furosemide in the diet for groups of 50 F344/N rats of each sex. Groups of 50 B6C3F$_1$ mice of each sex were fed diets containing 0, 700, or 1,400 ppm furosemide for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed and control rats were comparable throughout the studies. No significant differences in survival were observed between any groups of rats of either sex (final survival – male: control, 17/50; low dose, 17/50; high dose, 20/50; female: 35/50; 31/50; 34/50). The final survival of all groups of male rats was low, reflecting the large number of moribund animals killed after week 91. Survival at week 90 was 35/50, 28/50, and 34/50. Mean body weights of high dose male mice were up to 17% lower than those of controls, and mean body weights of low dose male mice were about 5%-10% lower than those of controls after week 31. Mean body weights of high dose female mice were up to 22% lower than those of controls. Mean body weights of low dose female mice were 5%-13% lower than those of controls

after week 82. The survival of the high dose group of female mice was significantly lower than that of controls after week 99 (final survival—male: 31/50; 24/50; 26/50; female: 36/50; 29/50; 18/50). Feed consumption by dosed rats was similar to that by controls. The estimated average amount of furosemide consumed per day was approximately 14-16 or 29-31 mg/kg for low dose or high dose rats. Feed consumption by dosed mice was approximately 5%-7% greater than that by controls. The average amount of furosemide consumed per day was approximately 91-99 or 191-214 mg/kg for low dose or high dose mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred at similar incidences in all groups of rats, but the severity was greater in dosed male rats. Tubular cell hyperplasia was observed in 4/50 control, 2/50 low dose, and 4/50 high dose male rats. Tubular cell adenomas of the kidney occurred in 1/50 control, 3/50 low dose, and 1/50 high dose male rats. Tubular cell adenocarcinomas were seen in a fourth low dose male rat and in a second high dose male rat (adenomas or adenocarcinomas, combined: control, 1/50; low dose, 4/50; high dose, 2/50). The historical incidence of renal tubular cell adenomas or adenocarcinomas (combined) in untreated male F344/N rats is 9/1,928 (0.5%), and the highest incidence observed in controls is 3/50.

Malignant meningiomas of the brain occurred in 3/50 low dose male rats; none was observed in other groups. The historical incidence of meningiomas in untreated male F344/N rats is 2/1,928 (0.1%).

C-Cell adenomas of the thyroid gland in female rats occurred with a positive trend; the incidence in the high dose group was not statistically greater than that in the controls (4/50; 6/50; 11/50). A C-cell carcinoma occurred in another low dose female rat. The incidence of adenomas of the anterior pituitary gland in low dose male rats was marginally greater than that in controls (4/50; 11/50; 8/50). Neither of these marginal increases was considered to be chemically related.

Malignant mixed tumors (adenocarcinoma, type C) of the mammary gland occurred in dosed female mice (0/50; 1/50; 5/48). One mammary gland acinar cell carcinoma occurred in a second low dose female mouse. The historical incidence of all malignant mammary gland neoplasms in untreated female B6C3F$_1$ mice is 40/2,040 (2%).

Compound-related nonneoplastic lesions of the kidney in mice included nephropathy and dilatation of the renal pelvis for males and females and tubular cysts, suppurative inflammation, and epithelial hyperplasia of the renal pelvis for males. Kidney lesions may have contributed to the low survival of high dose female mice.

Mucosal epithelial hyperplasia and submucosal chronic focal inflammation of the urinary bladder were observed at increased incidences in dosed male mice. Suppurative inflammation of the prostate was observed at an increased incidence in high dose male mice. Fighting may have contributed to urogenital lesions in male mice. Suppurative inflammation of the ovary or uterus was observed at an increased incidence in high dose female mice. Hematopoiesis was observed at increased incidences in the spleen and liver of dosed male and high dose female mice and in the adrenal cortex of high dose female mice.

Genetic Toxicology: Furosemide was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation. In the mouse lymphoma assay for trifluorothymidine (Tft) resistance, furosemide produced an equivocal response in the absence of metabolic activation and a positive response in the presence of activation. Furosemide induced sister chromatid exchanges and chromosomal aberrations in CHO cells in both the presence and absence of exogenous metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of furosemide have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusion: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of furosemide for male F344/N rats, as shown by marginal increases in uncommon tubular cell neoplasms of the kidney and meningiomas of the brain. There was *no evidence of carcinogenic activity* of furosemide for female F344/N rats fed diets containing 350 or 700 ppm furosemide for 2 years. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice fed diets containing 700 or 1,400 ppm furosemide for 2 years. There was *some evidence of carcinogenic activity* of furosemide for female mice, as shown by an increase in malignant tumors of the mammary gland.

Nephropathy was more severe in the kidney of male rats and of male and female mice fed diets containing furosemide than in controls.

Synonyms: 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid; frusemide; fursemide

Trade Names: Aisemide; Aluzine; Beronald; Desdemin; Diural; Dryptal; Errolon; Frusemin; Fulsix; Fuluvamide; Furosemide "Mita"; Katlex; Lasilix; Lasix; Lowpstron; Rosemide; Transit; Urosemide

Report Date: May 1989

TR-357 Toxicology and Carcinogenesis Studies of Hydrochlorothiazide (CAS No. 58-93-5) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Hydrochlorothiazide is a diuretic active at the distal convoluted tubule and collecting duct. Toxicology and carcinogenesis studies were conducted by feeding diets containing hydrochlorothiazide (USP grade, greater than 98% pure) to groups of F344/N rats and B6C3F$_1$ mice of each sex for 15 days, 13 weeks, 1 year, or 2 years. Additional studies were performed to evaluate teratologic effects in CD® rats and CD®-1 mice. Genetic toxicology studies were performed with Salmonella, Chinese hamster ovary (CHO) cells, mouse lymphoma cells, and Drosophila.

Fifteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 15-day studies (dietary concentrations of 0 and 3,125-50,000 ppm). The final mean body weights of all dosed rat groups were 5%-11% lower than those of controls. The final mean body weights of the groups of male mice that received 6,250-50,000 ppm were 10%-14% lower than that of controls. The final mean body weights of dosed and control female mice were similar. Calculi were seen in the urinary bladder of 2/5 male and 2/5 female mice at 50,000 ppm and in 1/5 male and 1/5 female mice at 25,000 ppm.

All rats lived to the end of the first 13-week studies (dietary concentrations of 0 and 3,125-50,000 ppm). Final body weights of dosed rats were 7%-16% lower than those of controls. Mineralization in the kidney was observed in all dosed rats and because of this, additional 13-week studies in rats were conducted at lower dietary concentrations. All rats lived to the end of the second 13-week studies (dietary concentrations of 0 and 250-4,000 ppm). The final mean body weights of all dosed rat groups were 5%-10% lower than those of controls. Renal mineralization was dose related and judged to be minimal to mild at the lowest dose.

In the 13-week studies in mice, 7/10 males and 1/10 females that received 50,000 ppm hydrochlorothiazide died. The final mean body weights of mice that received 50,000 ppm were 11% lower than those of controls for males and females. Calculi were seen in the urinary bladder of mice that received hydrochlorothiazide at 12,500 ppm and above. Nephrosis occurred with dose-related incidences in mice receiving 12,500 ppm and above.

Based on these results, 2-year studies were conducted by feeding diets containing 0, 250, 500, or 2,000 ppm hydrochlorothiazide to groups of 50 male and 50 female rats for 105-106 weeks. Diets containing 0, 2,500, or 5,000 ppm hydrochlorothiazide were fed to groups of 50 male and 50 female mice for 103-104 weeks. Ten additional rats per sex and dose group were placed on study and killed at 1 year for blood-clotting studies and histopathologic examination.

Effects in the One-Year Studies: One of 10 female rats in the 1-year study group that received 2,000 ppm died with internal hemorrhage. In addition, evidence of hemorrhage was found in 11 of the 16 dosed female rats that died during the first year of the 2-year study. Hematologic analyses revealed no compound-related effects; however, activated partial thromboplastin times (APTTs) were highly variable and were lengthened in some dosed male rats. No effects on APTTs were seen for females, and no effects on prothrombin times or on the fibrinogen content of plasma were observed for dosed male or female rats. Nephropathy occurred in dosed and control rats, and the severity was judged to be greater in dosed male and high dose female rats. Increased incidences of mild focal renal mineralization were also seen in mid and high dose male rats and dosed female rats.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were 8%-25% lower than those of controls. Mean body weights of dosed and control mice were similar throughout the studies. No significant differences in survival were observed between rats or mice of either sex (rats—male: control, 18/50; low dose, 16/50; mid dose, 9/50; high dose, 11/50; female: 31/50; 26/50; 30/50; 27/50; mice—male: control, 43/50; low dose, 42/50; high dose, 43/50; female: 38/50; 40/50; 35/50). Survival of all groups of male rats was low because a large number of animals were killed in a moribund condition late in the study. The average daily feed consumption by dosed rats was 89%-94% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 11, 23, or 89 mg/kg for low, mid, or high dose rats. The average daily feed consumption by dosed mice was 100%-105% that by controls. The average amount of hydrochlorothiazide consumed per day was approximately 280 or 575 mg/kg for low dose or high dose mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred in nearly all male and female rats, but the severity of this disease was greater in dosed rats, as evidenced by increases in renal cysts and epithelial hyperplasia of the renal pelvis in dosed rats shown in the following table (see page 4 of the Technical Report). Mineralization was observed at increased incidences in dosed male and dosed female rats.

Changes associated with or secondary to renal injury were increased in dosed rats. These lesions included parathyroid hyperplasia, fibrous osteodystrophy of bone, and mineralization of multiple organs.

Adenomas or carcinomas (combined) of the Zymbal gland in male rats occurred in 1/50 control, 1/49 low dose, 2/50 mid dose, and 4/50 high dose animals. The historical incidence of Zymbal gland neoplasms in untreated F344/N rats is 19/1,936 (1.0%), and the highest observed control group incidence is 4/50. This marginal increase was not considered to be chemically related.

The incidences of fibroadenomas of the mammary gland were decreased in dosed female rats (30/50; 12/50; 11/49; 5/50).

The incidence of hepatocellular neoplasms was increased in high dose male mice (adenomas or carcinomas, combined: control, 7/48; low dose, 10/49; high dose, 21/50). The historical incidence of hepatocellular adenomas or carcinomas (combined) is 609/2,032 (30%) in untreated controls.

Teratology: Hydrochlorothiazide produced no teratologic effects in the offspring of CD® rats or CD®-1 mice after gavage administration to pregnant females on day 6 through day 15 of gestation.

Genetic Toxicology: In the absence of exogenous metabolic activation, hydrochlorothiazide produced an equivocal increase in revertant colonies in *Salmonella typhimurium* strain TA98; no increase was observed in strains TA100, TA1535, or TA1537 with or without activation. Hydrochlorothiazide induced an increase in trifluorothymidine (Tft)-resistant cells in a mouse lymphoma L5178Y/TK$^{+/-}$ assay without exogenous metabolic activation; this assay was not performed with activation. In cultured CHO cells, hydrochlorothiazide induced sister chromatid exchanges (SCEs) in the presence and absence of exogenous metabolic activation but did not induce chromosomal aberrations. Hydrochlorothiazide did not increase the frequency of sex-

linked recessive lethal mutations when administered by feeding or injection to adult male *Drosophila melanogaster*.

Audit: The data, documents, and pathology materials from the 2-year studies of hydrochlorothiazide have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of hydrochlorothiazide for male or female F344/N rats given feed containing 250, 500, or 2,000 ppm hydrochlorothiazide. There was *equivocal evidence of carcinogenic activity* of hydrochlorothiazide for male $B6C3F_1$ mice, based on increased incidences of hepatocellular neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice given diets containing 2,500 or 5,000 ppm hydrochlorothiazide.

Chronic renal disease was more severe in rats administered hydrochlorothiazide, and increased incidences of secondary lesions (parathyroid hyperplasia, fibrous osteodystrophy, and mineralization in multiple organs) occurred in dosed rats.

Synonym: 6-chloro-3,4-dihydro-*2H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide

Trade Names: Aquarius; Bremil; Chlorzide; Cidrex; Dichlorosal; Dichlotride; Diclotride; Direma; Disalunil; Esidrix; Fluvin; Hidroronol; Hydril; Hydro-Aquil; Hydro-Diuril; Hydrosaluric; Hydrothide; Hypothiazide; Ivaugan; Jen-Diril; Maschitt; Nefrix; Neo-Codema; Neoflumen; Oretic; Panurin; Ro-Hydrazide; Thiaretic; Thiuretic; Urodiazin; Vetidrex

Report Date: July 1989

TR-358 Toxicology and Carcinogenesis Studies of Ochratoxin A (CAS No. 303-47-9) in F344/N Rats (Gavage Studies)

Ochratoxin A is a naturally occurring fungal toxin that is a contaminant in corn, peanuts, storage grains, cottonseed, meats, dried fish, and nuts. Toxicology and carcinogenesis studies were conducted by administering ochratoxin A (98% pure) in corn oil by gavage to groups of F344/N rats of each sex for 16 days, 13 weeks, 9 months, 15 months, or 2 years. Only rats were studied because ochratoxin A has been shown to be carcinogenic in mice. Genetic toxicology tests were performed with bacterial and mammalian cells. Urinalysis, hematologic and serum chemical analyses, and bone marrow cellularity determinations were conducted at 9, 15, and 24 months in the 2-year studies.

Sixteen-Day and Thirteen-Week Studies: Rats were administered 0, 1, 4, or 16 mg/kg ochratoxin A in corn oil by gavage 5 days per week for a total of 12 doses over 16 days. All rats that received 16 mg/kg ochratoxin A died within 6 days. Rats that received 4 mg/kg lost weight. Compound-related lesions in rats included bone marrow hyperplasia, thymic atrophy, necrosis and hyperplasia of the forestomach epithelium, renal tubular cell degenerative and regenerative changes (nephropathy), and adrenal gland hemorrhage. Renal tubular changes were most severe in animals that received 4 mg/kg. Rats that received 16 mg/kg had less severe renal lesions than those at 4 mg/kg, perhaps because the acute toxicity and early death did not allow sufficient time for full development of lesions.

No compound-related deaths occurred in the 13-week studies (doses were 0 and 0.0625 to 1 mg/kg). The final mean body weight of rats that received 0.25, 0.5, or 1 mg/kg was 7%, 11%, or 19% lower than that of vehicle controls for males and 3%, 4%, or 9% lower for females. Compound-related lesions in the kidney were characterized as degeneration and regeneration of the epithelium of the proximal convoluted tubules with individual cell necrosis of moderate severity (see page 3 of the Technical Report).

Karyomegaly of tubular epithelial cells was widespread but most pronounced in the straight portion of the tubules just above the corticomedullary junction. Karyomegaly was present in all dosed groups, and the severity increased as the dose increased. At lower doses, atrophy of the straight portions of the tubules at the corticomedullary junction and in the medulla was observed.

Based on mortality and on the presence and severity of renal lesions, groups of 80 rats per sex and dose group were administered 0, 21, 70, or 210 μg/kg ochratoxin A in corn oil by gavage 5 days per week for up to 2 years. Groups of 15 rats per sex and dose were killed at 9 or at 15 months and the remaining animals at 2 years.

Nine-Month and Fifteen-Month Studies: Administration of ochratoxin A by gavage for 9 months or 15 months to F344/N rats was associated with increased incidences of renal tubular cell neoplasms in males and hyperplasia, degeneration, and karyomegaly of renal tubular epithelial cells in both males and females (see page 4 of the Technical Report).

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were generally 4%-7% lower than those of vehicle controls. No significant differences in survival were observed between any groups of female rats (vehicle control, 32/50; low dose, 23/51; mid dose, 35/50; high dose, 34/50). Survival was decreased after 77 weeks in high dose male rats and after 96 weeks in low and mid dose male rats (39/50; 26/51; 26/51; 23/50).

Clinical Pathology: Minor differences were observed for hematologic values between dosed and vehicle control animals, but these were not considered to be of biologic significance. Results of serum chemistry analysis were not clearly compound related. Ochratoxin A-dosed animals had slight increases compared with vehicle controls in urine volume and decreases in urine specific gravity in concentration tests, suggesting that exposure resulted in mild to moderate decreases in the ability to concentrate urine.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: A spectrum of degenerative and proliferative changes occurred in the kidney of male and female rats given ochratoxin A for 2 years. Degeneration of the renal tubular epithelium with formation of tubular cysts, proliferation of the tubular epithelium, and karyomegaly of the nuclei of tubular epithelial cells occurred at increased incidences in dosed rats (see page 5 of the Technical Report). Hyperplasia of the renal tubular epithelium and renal tubular adenomas and carcinomas also occurred at increased incidences in the dosed rats; the tumors were frequently multiple within a single kidney or were bilateral, and many metastasized to other organs.

The incidence of fibroadenomas of the mammary gland in high dose female rats was significantly greater than that in vehicle controls (vehicle control, 17/50; low dose, 23/51; mid dose, 22/50; high dose, 28/50). Multiple fibroadenomas of the mammary gland were observed at an increased incidence in high dose female rats (4/50; 4/51; 5/50; 14/50). One mammary gland adenoma was seen in a mid dose female, and two mammary gland adenocarcinomas were seen in each dosed group; one adenocarcinoma was seen in the vehicle control group.

An adenoma of the pars intermedia of the pituitary gland was observed in one mid dose female rat, and a carcinoma was observed in a second mid dose female rat. Squamous cell papillomas of the tongue were seen in two low dose and two mid dose male rats. Neither the pituitary neoplasms nor the papillomas of the tongue were considered related to ochratoxin A exposure.

Genetic Toxicology: Ochratoxin A was not mutagenic in four strains of *Salmonella typhimurium* (TA97, TA98, TA100, or TA1535) when tested both with and without exogenous metabolic activation. In cultured Chinese hamster ovary (CHO) cells, ochratoxin A induced sister chromatid exchanges (SCEs) in the presence, but not the absence, of metabolic activation; it did not significantly increase the number of chromosomal aberrations in these cells.

Audit: The data, documents, and pathology materials from the 2-year studies of ochratoxin A have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of ochratoxin A for male F344/N rats as shown by substantially increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney. There was *clear evidence of carcinogenic activity* for female F344/N rats shown by increased incidences of uncommon tubular cell adenomas and of tubular cell carcinomas of the kidney and by increased incidences and multiplicity of fibroadenomas of the mammary gland.

Ochratoxin A administration also caused nonneoplastic renal changes including tubular cell hyperplasia, tubular cell proliferation, cytoplasmic alteration, karyomegaly, and degeneration of the renal tubular epithelium.

Synonym: (*R*)-*N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)-carbonyl](-L-)phenylalanine

Report Date: May 1989

TR-359 Toxicology and Carcinogenesis Studies of 8-Methoxypsoralen (CAS No. 298-81-7) in F344/N Rats (Gavage Studies)

Oral administration of 8-methoxypsoralen followed by exposure to longwave ultraviolet light (primarily ultraviolet A, 320-400 nm) is used in the treatment of vitiligo and psoriasis. 8-Methoxypsoralen also occurs naturally in a variety of vegetables. Toxicology and carcinogenesis studies of 8-methoxypsoralen without ultraviolet A were conducted by administering USP-grade 8-methoxypsoralen (99% pure) in corn oil by gavage to groups of F344/N rats once or for 16 days, 13 weeks, or 2 years. In vitro genetic toxicology tests were performed with bacteria and mammalian cells.

Single-Administration, Sixteen-Day, and Thirteen-Week Studies: In the single-administration studies, the chemical was administered at doses of 0 and 63-1,000 mg/kg. Four of five male rats and 5/5 female rats that received 1,000 mg/kg 8-methoxypsoralen died within 2 days.

In the 16-day studies, the chemical was administered at doses of 0 and 50-800 mg/kg. All rats receiving 800 mg/kg died within 5 days, and one male and one female at 400 mg/kg and one female at 200 mg/kg also died before the end of the studies. The final mean body weights of animals at 200 or 400 mg/kg were 14% or 30% lower than those of vehicle controls. No compound-related effects were observed at necropsy.

In the 13-week studies, the chemical was administered at doses of 0 and 25-400 mg/kg. Six of 10 male rats and 8/10 female rats that received 400 mg/kg died before the end of the studies. The final mean body weight of male rats that received 100, 200, or 400 mg/kg was 12%, 22%, or 45% lower than that of vehicle controls. The final mean body weight of female rats that received 200 or 400 mg/kg was 15% or 35% lower than that of vehicle controls. The liver weight to body weight ratios for all dosed groups of rats except the lowest (25 mg/kg) were greater than those for vehicle controls. Compound-related effects included fatty change in the liver in males and females and atrophy of the testis, seminal vesicles, and prostate.

Based on these results, 2-year studies were conducted by administering 0, 37.5 or 75 mg/kg 8-methoxypsoralen in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 F344/N rats of each sex.

Body Weight and Survival in the Two-Year Studies: The mean body weights of dosed male rats were generally 3%-14% lower than those of vehicle controls, and the mean body weights of high dose female rats were 5%-17% lower. The survival of both the low and the high dose groups of male rats was lower than that of the vehicle controls (male: vehicle control, 30/50; low dose, 16/50; high dose, 16/50; female: 39/50; 33/50; 36/50), likely because of kidney toxicity and neoplasia.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Mineralization of the renal papilla was observed in high dose male rats (vehicle control, 0/50; low dose, 0/50; high dose, 31/49). The severity of nephropathy was increased in dosed male rats. Focal hyperplasia of renal tubular cells was observed in dosed male rats (0/50; 8/50; 8/49). The incidences of tubular cell adenomas (1/50; 11/50; 8/49), adenocarcinomas (0/50; 1/50; 3/49), and adenomas or adenocarcinomas (combined) (1/50; 12/50; 11/49) were increased in dosed male rats. Hyperplasia of the parathyroid glands (2/49; 22/47; 18/48) and fibrous osteodystrophy (2/50; 10/50; 12/49) in male rats were secondary to chronic nephropathy.

The incidences of carcinomas or squamous cell carcinomas (combined) of the Zymbal gland were increased in dosed male rats (1/50; 7/50; 4/49). The mean historical incidence for carcinomas or squamous cell carcinomas (combined) in corn oil vehicle control male F344/N rats is 0.8% (16/1,949); the highest incidence in any one group is 4% (2/49).

Fibromas of the subcutaneous tissue in male rats occurred with a positive trend (1/50; 5/50; 7/49). An additional high dose male had a sarcoma. The mean historical incidence of fibromas or fibrosarcomas (combined) of subcutaneous tissue in corn oil vehicle control male F344/N rats is 9% (171/1,949).

Alveolar/bronchiolar adenomas occurred with a positive trend in male rats (4/50; 9/50; 9/49).
The mean historical incidence of alveolar/bronchiolar neoplasms in corn oil vehicle control male F344/N rats is 3% (68/1,944); the highest incidence is 10% (5/50).

Chronic inflammation, ulcers, and epithelial hyperplasia of the forestomach were observed at increased incidences in dosed male rats (chronic inflammation: 1/50; 6/50; 5/49; ulcers: 5/50; 13/50; 11/49; epithelial hyperplasia: 4/50; 19/50; 20/49). Squamous cell papillomas were observed in two low dose male rats.

Squamous cell papillomas were observed in the palate or tongue of one low dose and three high dose female rats; none were observed in vehicle controls. These papillomas were not considered to be related to chemical administration.

Diffuse hypertrophy of the thyroid gland was observed at increased incidences in dosed male rats (2/50; 31/50; 39/49).

Genetic Toxicology: 8-Methoxypsoralen was mutagenic in *Salmonella typhimurium* strain TA104 in the presence and absence of activation and in strains TA98, TA100, and TA102 when tests were conducted with exogenous metabolic activation; 8-methoxypsoralen was not mutagenic with or without activation in strain TA1535. Treatment with 8-methoxypsoralen induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of exogenous metabolic activation; in the presence of activation, in the presence of activation, induction of SCEs occurred, but no significant increases in chromosomal aberrations was observed.

Audit: The data, documents, and pathology materials from the 2-year studies of 8-methoxypsoralen have been audited at the NTP Archives. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of 8-methoxypsoralen (without ultraviolet radiation) for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male F344/N rats may have been related to chemical administration. Dose-related nonneoplastic lesions in male F344/N rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was *no evidence of carcinogenic activity* of 8-methoxypsoralen for female F344/N rats given the chemical at 37.5 or 75 mg/kg per day for 2 years.

Synonyms: 9-methoxy-*7H*-furo[3,2-*g*]benzopyran-7-one; 6-hydroxy-7-methoxy-5-benzofuranacrylic acid 6-lactone; 8-MP; 8-MOP; 8-methoxy-(furano-3',2':6,7-coumarin); 8-methoxy-4',5':6,7-furocoumarin; 9-methoxypsoralen; 8-methoxypsoralene; methoxsalen; oxypsoralen

Trade Names: Ammoidin; Meladinin (VAN); Meladinine; Meladoxen; Meloxine; Methoxa-Dome; Mopsoralen; Oxsoralen; Soloxsalen; Trioxun; Xanthotoxin; Xanthotoxine

Report Date: July 1989

TR-360 Toxicology and Carcinogenesis Studies of *N,N*-Dimethylaniline (CAS No. 121-69-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

N,N-Dimethylaniline is used as a chemical intermediate in the synthesis of dyestuffs. Toxicology and carcinogenesis studies were conducted by administering *N,N*-dimethylaniline (greater than 98% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 2 weeks, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Two-Week and Thirteen-Week Studies: In the 2-week studies, doses were 94-1,500 mg/kg; deaths of rats and mice were observed in groups given doses of 750 or 1,500 mg/kg. The final mean body weights of male rats that received 375 or 750 mg/kg were 15% or 47% lower than that of vehicle controls; final mean body weights of other groups of rats and mice were similar to those of vehicle controls. Compound-related clinical signs observed included cyanosis in rats and lethargy and tremors in rats and mice. Splenomegaly occurred in nearly all dosed groups of rats and mice, and the incidences were dose related.

In the 13-week studies, doses were 32-500 mg/kg; no compound-related deaths occurred. The final mean body weights of male rats that received 250 or 500 mg/kg were 15% or 27% lower than that of vehicle controls. The final mean body weights of all groups of dosed female rats and male and female mice were within 12% of those of vehicle controls. Compound-related clinical signs included lethargy in rats and mice and cyanosis in rats. Splenomegaly was observed in all dosed groups of rats and mice; the severity was dose related. Compound-related extramedullary hematopoiesis and hemosiderosis occurred in the kidney or testis of dosed rats and liver and spleen of dosed rats and mice.

Two-year studies were conducted by administering 0, 3, or 30 mg/kg *N,N*-dimethylaniline in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 rats of each sex. The lower dose was selected to be one-tenth the higher dose to increase the likelihood that one dose would cause only a minimal nonneoplastic response. Groups of 50 mice of each sex were administered 0, 15, or 30 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of vehicle control and dosed rats and mice were similar throughout the studies. Survival rates of all respective groups were similar after 2 years, except for the lowered survival of vehicle control female rats (vehicle control, 21/50; low dose 32/50; high dose, 36/50). This may reflect the large number (24/50) of vehicle control female rats killed when observed to be in a moribund state. Final survival for other groups was as follows: male rats—29/50; 32/50; 28/50; male mice—34/50; 30/50; 34/50; female mice—35/50; 39/50; 33/50.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In these 2-year studies, the spleen was the expected site of chemical-related effects. Fatty metamorphosis and fibrosis in the spleen of high dose male rats were increased (fatty metamorphosis: vehicle control, 0/49; low dose, 1/49; high dose, 10/50; fibrosis: 5/49; 2/49; 22/50). Splenic hemosiderosis and hematopoiesis were present at an incidence greater than 85% in all groups of rats; however, the severity of the lesions was greater in dosed groups than in vehicle controls. Sarcomas of the spleen were seen in 3/50 high dose male rats, and an osteosarcoma was seen in another high dose male rat. One additional high dose male rat had a sarcoma of the thymus. Splenic sarcomas are uncommon in corn oil vehicle control male F344/N rats (NTP historical incidence 3/2,081, 0.1%), and thus, these neoplasms in high dose male rats (4/50, 8%) were considered to be chemically related.

Lower incidences of mononuclear cell leukemia (which apparently originates in the spleen) were seen in dosed male and female rats than in vehicle controls (male: 13/50; 4/50; 3/50; female: 11/50; 7/50; 0/50).

The incidence of squamous cell papillomas of the forestomach in high dose female mice was marginally greater than that in vehicle controls (2/50; 2/50; 8/50). No malignant forestomach neoplasms were observed.

Genetic Toxicology: *N,N*-Dimethylaniline was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of exogenous metabolic activation. In the mouse lymphoma assay, *N,N*-dimethylaniline produced a positive response with and without metabolic activation. In CHO cells, *N,N*-dimethylaniline induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in the presence of exogenous metabolic activation. Without activation, an increase in chromosomal aberrations was observed, but no increase in SCEs occurred.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of *N,N*-dimethylaniline for male F344/N rats, as indicated by the increased incidences of sarcomas or osteosarcomas (combined) of the spleen. There was *no evidence of carcinogenic activity* of *N,N*-dimethylaniline for female F344/N rats given 3 or 30 mg/kg body weight by gavage for 2 years. There was *no evidence of carcinogenic activity* of *N,N*-dimethylaniline for male $B6C3F_1$ mice given 15 or 30 mg/kg body weight by gavage for 2 years. There was *equivocal evidence of carcinogenic activity* of *N,N*-dimethylaniline for female $B6C3F_1$ mice, as indicated by an increased incidence of squamous cell papillomas of the forestomach. Both rats and mice could have tolerated doses higher than those used in these studies.

There were decreased incidences of mononuclear cell leukemia in dosed male and high dose female rats. Compound-related splenic fibrosis, hemosiderosis, and fatty metamorphosis were increased in male rats.

Synonyms: dimethylaminobenzene; *N,N*-dimethylbenzeneamine; dimethylaniline; dimethylphenylamine; *N,N*-dimethylphenylamine

Report Date: October 1989

TR-361 Toxicology and Carcinogenesis Studies of Hexachloroethane (CAS No. 67-72-1) in F344/N Rats (Gavage Studies)

Hexachloroethane is used in organic synthesis as a retarding agent in fermentation, as a camphor substitute in nitrocellulose, in pyrotechnics and smoke devices, in explosives, and as a solvent. In previous long-term gavage studies with $B6C3F_1$ mice and Osbourne-Mendel rats (78 weeks of exposure followed by 12-34 weeks of observation), hexachloroethane caused increased incidences of hepatocellular carcinomas in mice. However, survival of low and high dose rats was reduced compared with that of vehicle controls, and the effects on rats were inconclusive. Therefore, additional toxicology and carcinogenesis studies were conducted in F344/N rats by administering hexachloroethane (approximately 99% pure) in corn oil by gavage to groups of males and females for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary (CHO)

cells. Urinalysis was performed in conjunction with the 13-week studies.

Sixteen-Day Studies: In the 16-day studies (dose range, 187-3,000 mg/kg), all rats that received 1,500 or 3,000 mg/kg and 1/5 males and 2/5 females that received 750 mg/kg died before the end of the studies. Final mean body weights of rats that received 750 mg/kg were 25% lower than that of vehicle controls for males and 37% lower for females. Compound-related clinical signs seen at 750 mg/kg or more included dyspnea, ataxia, prostration, and excessive lacrimation. Other compound-related effects included hyaline droplet formation in the tubular epithelial cells in all dosed males and tubular cell regeneration and granular casts in the tubules at the corticomedullary junction in the kidney in males receiving 187 and 375 mg/kg.

Thirteen-Week Studies: In the 13-week studies (dose range, 47-750 mg/kg), 5/10 male rats and 2/10 female rats that received 750 mg/kg died before the end of the studies. The final mean body weight of male rats that received 750 mg/kg was 19% lower than that of vehicle controls. Compound-related clinical signs for both sexes included hyperactivity at doses of 94 mg/kg or higher and convulsions at doses of 375 or 750 mg/kg. The relative weights of liver, heart, and kidney were increased for exposed males and females. Kidney lesions were seen in all dosed male groups, and the severity increased with dose. Papillary necrosis and tubular cell necrosis and degeneration in the kidney and hemorrhagic necrosis in the urinary bladder were observed in the five male rats that received 750 mg/kg and died before the end of the studies; at all lower doses, hyaline droplets, tubular regeneration, and granular casts were present in the kidney. No chemical-related kidney lesions were observed in females. Foci of hepatocellular necrosis were observed in several male and female rats at doses of 188 mg/kg or higher.

Dose selection for the 2-year studies was based primarily on the lesions of the kidney in males and of the liver in females. Studies were conducted by administering hexachloroethane in corn oil by gavage at 0, 10, or 20 mg/kg body weight, 5 days per week, to groups of 50 male rats. Groups of 50 female rats were administered 0, 80, or 160 mg/kg on the same schedule.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were slightly (5%-9%) lower than those of vehicle controls toward the end of the studies. No significant differences in survival were observed between any groups of rats (male: vehicle control, 31/50; 10 mg/kg, 29/50; 20 mg/kg, 26/50; female: vehicle control, 32/50; 80 mg/kg, 27/50; 160 mg/kg, 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Incidences of kidney mineralization (vehicle control, 2/50; low dose, 15/50; high dose, 32/50) and hyperplasia of the pelvic transitional epithelium (0/50; 7/50; 7/50) were increased in dosed male rats. Renal tubule hyperplasia was observed at an increased incidence in high dose male rats (2/50; 4/50; 11/50). These lesions have been described as characteristic of the hyaline droplet nephropathy that is associated with an accumulation of liver-generated $\alpha_2\mu$-globulin in the cytoplasm of tubular epithelial cells. The severity of nephropathy was increased in high dose male rats (moderate vs. mild), and the incidences and severity of nephropathy were increased in dosed females (22/50; 42/50; 45/50). The incidences of adenomas (1/50; 2/50; 4/50), carcinomas (0/50; 0/50; 3/50), and adenomas or carcinomas (combined) (1/50; 2/50; 7/50) of the renal tubule were also increased in the high dose male group. One of the carcinomas in the high dose group metastasized to the lung. No compound-related neoplasms were observed in females.

The incidence of pheochromocytomas of the adrenal gland in low dose male rats was significantly greater than that in vehicle controls (15/50; 28/50; 21/49), and the incidences for both dosed groups were greater than the mean historical control incidence (28% ± 11%).

Genetic Toxicology: Hexachloroethane was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with and without exogenous metabolic activation. In CHO cells, hexachloroethane did not induce chromosomal aberrations with or with out metabolic activation but did produce sister chromatid exchanges in the presence of exogenous metabolic activation.

Audit: The data, documents, and pathology materials from the 2-year studies of hexachloroethane have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of hexachloroethane for male F344/N rats, based on the increased incidences of renal neoplasms. The marginally increased incidences of pheochromocytomas of the adrenal gland may have been related to hexachloroethane administration to male rats. There was *no evidence of carcinogenic activity* of hexachloroethane for female F344/N rats administered 80 or 160 mg/kg by gavage for 103 weeks.

The severity of nephropathy and incidences of linear mineralization of the renal papillae and hyperplasia of the transitional epithelium of the renal pelvis were increased in dosed male rats. The incidences and severity of nephropathy were increased in dosed female rats.

Synonyms: carbon hexachloride; ethane hexachloride; hexachlorethane; hexachloroethylene; 1,1,1,2,2,2-hexachloroethane; perchloroethane

Trade Names: Avlothane; Distokal; Distopan; Distopin; Egitol; Falkitol; Fasciolin; Mottenhexe; Phenohep

Report Date: August 1989

Note: Hexachloroethane was previously tested in Osborne-Mendel rats and B6C3F$_1$ mice by gavage (See TR-68, reported 1978).

TR-362 Toxicology and Carcinogenesis Studies of 4-Vinyl-1-cyclohexene Diepoxide (CAS No. 106-87-6) in F344/N Rats and B6C3F$_1$ Mice (Dermal Studies)

4-Vinyl-1-cyclohexene diepoxide is used a chemical intermediate and as a reactive diluent for diepoxides and epoxy resins. Toxicology and carcinogenesis studies were conducted by administering 4-vinyl-1-cyclohexene diepoxide (97% pure) in acetone by dermal application to individually housed F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, 15 months, and 2 years. Additional studies included evaluation of immune function after a 5-day dermal exposure and evaluation of the oral toxicity of 4-vinyl-1-cyclohexene diepoxide in 16-day and 13-week corn oil gavage studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Dermal Studies: In the 14-day studies, all rats that received 924 mg/kg or higher (equivalent to 139 mg/rat or higher for males and 112 mg/rat of higher for females) died before the end of the studies. Final mean body weights were lower than those of vehicle controls in males receiving 68 mg/rat and in females receiving 57 mg/rat. Excoriations on the skin at the application site were observed in the groups receiving 57 mg/rat or more. Males receiving 139 mg/rat and females receiving 112 mg/rat had congestion and/or hypoplasia of the bone marrow; most had acute nephrosis. Skin lesions, including epidermal necrosis and ulceration, epidermal hyperplasia, and hyperkeratosis, were found in the 139 and 112 mg/rat group; similar lesions of lesser severity were seen in the two lowest dose groups of each sex.

All mice that received 1,787 mg/kg (equivalent to 43/mouse for males and 37 mg/mouse for females) and 3/5 male mice and 5/5 female mice that received 889 mg/kg (equivalent to 19-21 mg/mouse) died before the end of the 14-day dermal studies. Final mean body weights of exposed and vehicle control mice were generally similar. Lesions of the skin at the site of application were seen in 4/5 males and 4/5 females receiving 5 mg/mouse and all mice receiving 10 and 21 (males) or 19 (females) mg/mouse and included epidermal and sebaceous gland hyperplasia, hyperkeratosis, and ulceration.

Thirteen-Week Studies: In the 13-week dermal studies, all rats survived to the end of the studies (doses up to 60 mg/rat). The final mean body weights of the 60 mg/rat groups were 9%-14% lower than those of the vehicle controls. Compound-related clinical signs in the 60 mg/rat groups observed during the second half of the studies included redness, scabs, and ulceration at the application site and burrowing behavior after dermal application. Hyperplasia of the sebaceous glands and acanthosis (hyperplasia) and hyperkeratosis of the squamous epithelium were seen at the site of application.

In mice, no compound-related deaths occurred after applications of up to 10 mg/mouse in 13-week dermal studies, and final mean body weights of exposed and vehicle control mice were similar. Relative liver and kidney weights increased with dose. Compound-related lesions of the skin included sebaceous gland hyperplasia and acanthosis (hyperplasia) and hyperkeratosis of the stratified squamous epithelium at the site of application; ovarian atrophy was also considered to be compound related.

In the 13-week oral studies, the major target organ of toxicity in rats and mice was the forestomach, as indicated by hyperkeratosis and hyperplasia of the stratified squamous epithelium. In female mice, ovarian atrophy was seen in 4-vinyl-1-cyclohexene diepoxide-dosed groups.

Two-year studies were conducted by administering 4-vinyl-1-cyclohexene diepoxide in acetone by dermal application, 5 days per week for 105 weeks to groups of 60 rats of each sex at 0, 15, or 30 mg/animal. Groups of 60 mice of each sex were administered 0, 2.5, 5, or 10 mg/animal on the same schedule for 103 weeks. None of the doses selected had produced ulceration of skin in 13-week studies. Ten animals from each group were killed and examined during month 15 for toxicologic evaluation.

Immune Function Studies: The immunotoxic effects of 4-vinyl-1-cyclohexene diepoxide were studied in male B6C3F$_1$ mice after a 5-day dermal exposure at doses ranging from 2.5 to 10 mg/mouse per day. 4-Vinyl-1-cyclohexene diepoxide was immunosuppressive at 10 mg/mouse and, to a lesser extent, at 5 mg/mouse, as indicated by a decrease in peripheral lymphocytes and the in vitro lymphoproliferative response to phytohemagglutinin and concanavalin A in the high dose group and suppression of the antibody plaque-forming-cell response in the 5 and 10 mg/mouse groups.

Fifteen-Month Evaluation: Two of 10 male rats that received 30 mg had a squamous cell carcinoma of the skin at or adjacent to the site of application. Acanthosis was seen in exposed rats (mild severity at 30 mg/rat and minimal severity at 15 mg/rat); hyperkeratosis was observed for rats in the 30 mg/rat groups.

Compound-related nonneoplastic skin lesions in mice included acanthosis, hyperkeratosis, and sebaceous gland hyperplasia/hypertrophy. Squamous cell papillomas and carcinomas were seen in mice that received 5 or 10 mg/mouse; none was seen in vehicle control or low dose groups (papillomas—male: mid dose, 1/10; high dose, 2/10; female: 1/10; 1/10; carcinomas—male: 2/10; 8/10; female: 2/10; 5/10). One vehicle control and all exposed female mice had atrophy of the ovary. Hyperplasia of the ovarian surface epithelium was seen in 8/10 females receiving 5 mg/mouse and 9/9 females receiving 10 mg/mouse. Two of nine females receiving 10 mg/mouse had granulosa cell tumors of the ovary, and 1/9 females receiving 10 mg/mouse had an ovarian papillary cystadenoma.

Body Weights and Survival in the Two-Year Studies: In general, the body weights and survival were lower in mid and high dose groups than in vehicle controls. The survival was lower in exposed groups, primarily because of neoplasms (survival at week 105—male rats: vehicle control, 7/50; low dose, 8/50; high dose, 4/50; female rats: 27/50; 23/50; 15/50; male mice: vehicle control, 38/50; low

dose, 35/50; mid dose, 4/50; high dose, 0/50; female mice: 30/50; 31/50; 15/50; 0/50). All high dose male mice died by week 83; the 10 surviving high dose female mice were killed during week 85.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Acanthosis and sebaceous gland hypertrophy of skin from the scapula or back were observed at substantially increased incidences in exposed male and female rats. Squamous cell papillomas in male rats and squamous cell carcinomas in male and female rats were observed only in exposed rats (squamous cell carcinomas – male: vehicle control, 0/50; low dose, 33/50; high dose, 36/50; female: 0/50; 16/50; 34/50). The incidences of basal cell adenomas or carcinomas (combined) were increased (male: 0/50; 1/50; 6/50; female: 0/50; 3/50; 4/50).

For exposed mice, acanthosis, hyperkeratosis, and necrotizing inflammation of the skin were observed over the scapula or back. Squamous cell carcinomas were found only in exposed mice (male: vehicle control, 0/50; low dose, 14/50; mid dose, 39/50; high dose, 42/50; female: 0/50; 6/50; 37/50; 41/50).

Follicular atrophy and tubular hyperplasia of the ovary in female mice were increased (atrophy: 12/50; 43/49; 47/50; tubular hyperplasia: 5/50; 35/49; 38/49; 34/50). Mid and high dose females had benign or malignant granulosa cell tumors (0/50; 0/49; 7/49; 12/50) and benign mixed tumors (0/50; 0/49; 11/49; 6/50). The combined incidences of luteomas, granulosa cell tumors, benign mixed tumors, or malignant granulosa cell tumors in mid and high dose female mice were increased (1/50; 0/49; 17/49; 18/50).

The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in exposed female mice were marginally increased (4/50; 9/50; 11/50; 7/50).

Genetic Toxicology: 4-Vinyl-1-cyclohexene diepoxide was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 with and without exogenous metabolic activation; the compound was equivocally mutagenic in strain TA1537 without S9 but gave a positive response in the presence of activation. 4-Vinyl-1-cyclohexene diepoxide induced resistance to trifluorothymidine in mouse L5178Y/TK cells without exogenous metabolic activation; it was not tested with activation. 4-Vinyl-1-cyclohexene diepoxide induced sister chromatid exchanges and chromosomal aberrations in CHO cells in the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year dermal studies, there was *clear evidence of carcinogenic activity* of 4-vinyl-1-cyclohexene diepoxide for male and female F344/N rats, as shown by squamous cell and basal cell neoplasms of the skin. There was *clear evidence of carcinogenic activity* of 4-vinyl-1-cyclohexene diepoxide for male and female $B6C3F_1$ mice, as shown by squamous cell carcinomas of the skin in males and squamous cell carcinomas of the skin and ovarian neoplasms in females; increased incidences of lung neoplasms in females may also have been related to chemical application.

Synonyms: 4-vinylcyclohexene diepoxide; 4-vinyl-1,2-cyclohexene diepoxide; 1-vinyl-3-cyclohexene diepoxide; 4-vinylcyclohexene dioxide; 1,2-epoxy-4-(epoxyethyl) cyclohexane; 1-epoxyethyl-3,4-epoxycyclohexane

Report Date: November 1989

TR-363 Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS No. 74-96-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Bromoethane is an alkylating agent used primarily as a chemical intermediate in various organic syntheses. In toxicology and carcinogenesis studies, groups of F344/N rats and $B6C3F_1$ mice of each sex received whole-body exposure to bromoethane (greater than 98% pure) once for 4 hours or for 6 hours per day, 5 days per week, for 14 days, 14 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Single-Exposure, Fourteen-Day, and Fourteen-Week Studies: Single-exposure inhalation studies were conducted in rats and mice at target concentrations of 625, 1,250, 2,500, 5,000, or 10,000 ppm bromoethane. All rats exposed to 10,000 ppm bromoethane and 3/5 female rats exposed to 5,000 ppm died before the end of the single-exposure studies. All mice exposed to 5,000 or 10,000 ppm bromoethane and 2/5 female mice exposed to 1,250 ppm died before the end of the studies.

Fourteen-day inhalation studies were conducted in rats and mice at target concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm bromoethane. All rats and mice exposed to 2,000 or 4,000 ppm died before the end of the 14-day studies. Final mean body weights of exposed and control rats were similar.

Fourteen-week inhalation studies were conducted in rats and mice at target concentrations of 0, 100, 200, 400, 800, or 1,600 ppm bromoethane. Four of 10 male and 2/10 female rats exposed to 1,600 ppm died before the end of the 14-week studies. The final mean body weights of rats exposed to 1,600 ppm were lower than the initial mean body weights. Compound-related lesions observed in rats at 1,600 ppm, but not at lower concentrations, included minimal atrophy of the thigh muscle, minimal-to-moderate multifocal mineralization in the cerebellum of the brain, minimal-to-severe hemosiderosis of the spleen, marked atrophy of the testis, and minimal-to-mild atrophy of the uterus. The effects in the testis and uterus are probably due to chemical-related loss in body weight during the studies.

In mice, compound-related deaths included 3/10 male and 1/10 female mice exposed to 1,600 ppm, 1/9 males exposed to 800 ppm, and 1/10 males exposed to 400 ppm. The final mean body weights of male and female mice exposed to 1,600 ppm were about 15% lower than those of controls. Compound-related effects included atrophy of the uterus and involution of the ovary in females exposed to 1,600 ppm bromoethane.

Based on these results, 2-year studies were conducted by exposing groups of 49 or 50 rats or mice of each sex to bromoethane at 0, 100, 200, or 400 ppm, 6 hours per day, 5 days per week.

Body Weight and Survival in the Two-Year Studies: Mean body weights of exposed and control rats were generally similar throughout the studies. No significant differences in survival were observed between any groups of male rats (control, 17/49; 100 ppm, 26/50; 200 ppm, 27/50; 400 ppm, 21/50); survival of the 100-ppm group of female rats was greater than that of controls (19/50; 29/50; 24/49; 23/50), and the number of control and 400-ppm male rats and control female rats surviving to the end of the studies was low.

Mean body weights of the 400-ppm group of male mice were up to 9% lower than those of controls throughout the study. Mean body weights of the 400-ppm group of female mice were generally 6%-16% lower than those of controls after week 29. No differences in survival were observed between any group of male mice (35/50; 37/50; 30/50; 34/50). The survival of the 400-ppm group of female mice was lower than that of controls at the end of the study (36/50; 37/50; 37/49; 23/49).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal medulla were increased in exposed male rats (control, 8/40; 100 ppm, 23/45; 200 ppm, 18/46; 400 ppm, 21/46).

Granular cell neoplasms of the brain were seen in exposed male rats but not in controls (0/49; 3/50; 1/50; 1/50). A glioma, an astrocytoma, or an oligodendroglioma was seen in 3/50 male rats exposed to 100 ppm. Gliomas were not observed in control female rats, but they occurred in exposed female rats with a significant positive trend (0/50; 1/50; 1/48; 3/50). The historical incidence of granular cell tumors in male F344/N rat chamber controls at the study laboratory is 0/297. The incidences of gliomas in the exposed female groups were not significantly greater than that in the controls and were within the historical incidence range for glial cell neoplasms for untreated controls in NTP studies (mean: 23/1,969, 1%; range: 0/50-3/50), but they exceeded the historical incidence range for chamber controls at the study laboratory (mean: 1/297, 0.3%; range: 0/50-1/50).

Alveolar epithelial hyperplasia was increased in rats exposed to 400 ppm bromoethane (male: 3/48; 7/49; 7/48; 18/48; female: 5/50; 4/48; 5/47; 10/49). Alveolar/bronchiolar adenomas or carcinomas (combined) were seen in four male rats exposed to 200 ppm and in one exposed to 400 ppm. Alveolar/bronchiolar adenomas were observed in 3/49 female rats at 400 ppm but not at lower concentrations or in controls. The incidences in exposed male and female rats were not significantly greater than those in controls; however, the historical incidence in rat chamber controls for alveolar/bronchiolar adenomas or carcinomas (combined) at the study laboratory is 6/299 (2%) for males and 4/297 (1.3%) for females.

The incidences of epithelial hyperplasia and squamous metaplasia of the nasal cavity were increased in rats exposed to 400 ppm. The incidence of suppurative inflammation of the nasal cavity was increased in exposed male rats, and the incidences of suppurative inflammation of the larynx and metaplasia of the olfactory sensory epithelium were increased in exposed male and female rats. An adenoma of the nose was seen in one 400-ppm male rat and in one 400-ppm female mouse.

Suppurative inflammation and dilatation of the salivary gland ducts were observed at increased incidences in the 200- and 400-ppm groups of female rats. Animals were found to be positive for rat coronavirus/sialodacryoadenitis virus antibodies.

The incidence of mammary gland neoplasms was significantly lower in female rats at 400 ppm than in controls (18/50; 15/50; 10/48; 7/50).

Adenomas (0/50; 1/50; 1/47; 6/48), adenocarcinomas (0/50; 2/50; 3/47; 19/48), and squamous cell carcinomas (0/50; 1/50; 1/47; 3/48) of the uterus occurred in exposed female mice and not in control mice.

The incidence of alveolar/bronchiolar neoplasms was greater in male mice at 400 ppm than in controls (adenomas or carcinomas, combined: 7/50; 6/50; 12/50; 15/50). Acute/chronic inflammation of the lung was observed at increased incidences in female mice at 200 and 400 ppm.

Genetic Toxicology: Bromoethane, tested in a closed environment of a desiccator, was mutagenic in *S. typhimurium* strain TA100 with and without exogenous metabolic activation; it was not mutagenic in strain TA98. In cultured CHO cells, bromoethane induced sister chromatid exchanges (SCEs) but not chromosomal aberrations in both the presence and absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of bromoethane for male F344/N rats, as indicated by increased incidences of pheochromocytomas of the adrenal gland; neoplasms of the brain and lung may also have been related to exposure to bromoethane. For female F344/N rats, there was *equivocal evidence of carcinogenic activity*, as indicated by marginally increased incidences of neoplasms of the brain and lung. For male $B6C3F_1$ mice, there was *equivocal evidence of carcinogenic activity*, based on marginally increased incidences of neoplasms of the lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, as indicated by neoplasms of the uterus.

Synonyms: monobromoethane; bromic ether; hydrobromic ether

Report Date: October 1989

TR-364 Toxicology and Carcinogenesis Studies of Rhodamine 6G (C.I. Basic Red 1) (CAS No. 989-38-8) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Toxicology and carcinogenesis studies of rhodamine 6G were conducted because of potential human exposure

related to its use as a dye for natural and synthetic fibers and as a research chemical. These studies were conducted by administering rhodamine 6G (greater than 95% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (0, 310, 620, 1,250, 2,500, or 5,000 ppm), all five male and five female rats that received 5,000 ppm and 1/5 male rats that received 2,500 ppm died before the end of the studies; all mice lived to the end of the study. The final mean body weights of rats that received 2,500 ppm were lower than the initial weights. The final mean body weights of mice that received 2,500 or 5,000 ppm were 8% or 18% lower than that of controls for males and 2% or 8% lower for females.

In the 13-week studies, all rats lived to the end of the studies (dietary concentrations of 0 or 120-2,000 ppm). The final mean body weights of rats that received 500, 1,000 or 2,000 ppm were 12%, 13%, or 32% lower than that of controls for males and 4%, 8%, or 20% lower for females. Feed consumption by rats that received 2,000 ppm was somewhat lower than that by controls. Bone marrow atrophy was observed at increased incidences and severity in dosed rats. In the 13-week study (0 or 500-8,000 ppm), 1/10 male mice that received the highest concentration died before the end of the studies. The final mean body weights of mice that received 8,000 ppm were lower than the initial mean body weights. The final mean body weights of male mice that received 4,000 ppm and of female mice that received 2,000 or 4,000 ppm were 13%-19% lower than those of controls. Feed consumption was not related to dose. Minimal-to-moderate cytoplasmic vacuolization of hepatocytes was seen in 8/10 male mice that received 8,000 ppm.

Based on these results, dietary concentrations selected for the 2-year studies were 0, 120, or 250 ppm rhodamine 6G for rats, 0, 1,000, or 2,000 ppm for male mice, and 0, 500, 1,000 ppm for female mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were similar to those of controls throughout the studies. The average daily feed consumption by dosed rats was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 5 mg/kg for low dose rats and 10 or 12 mg/kg for high dose male or female rats. Mean body weights of high dose male and dosed female mice were generally 5%-14% lower than those of controls. The average daily feed consumption by dosed mice was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 210 or 440 mg/kg for low dose or high dose male mice and 125 or 250 mg/kg for low dose or high dose female mice. No significant differences in survival were observed between any groups of rats or mice (male rats: control, 22/50; low dose, 21/50; high dose, 27/50; female rats: 29/50; 30/50; 30/50; male mice: 36/50; 32/50; 38/50; female mice: 39/50; 35/50; 36/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No chemically related nonneoplastic lesions in male or female rats and no chemically related neoplastic or nonneoplastic lesions in male or female mice were observed in these studies.

The incidences of keratoacanthomas of the skin was increased in high dose male rats (control, 1/50; low dose, 2/50; high dose, 8/50). The historical incidence of keratoacanthomas in untreated control male F344/N rats is 31/1,936 (1.6%; range, 0/50-7/49). Both fur and skin of rats in the dosed groups apparently were exposed to feed dust containing rhodamine 6G; the intensity of staining was proportional to the concentration of rhodamine 6G in feed. Because of the variable background incidence of keratoacanthomas in F344/N rats, the incidence of keratoacanthomas cannot be conclusively related to exposure to rhodamine 6G.

The incidences of pheochromocytomas (3/50; 3/50; 8/50) or malignant pheochromocytomas (combined: 3/50; 3/50; 10/50) of the adrenal gland were increased in high dose female rats. The historical incidence of adrenal medullary neoplasms in untreated control F344/N female rats is 99/1,968 (5%; range, 0/50-8/50). This marginal increase may be related to the administration of rhodamine 6G.

Genetic Toxicology: Rhodamine 6G was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with and without exogenous metabolic activation (S9). Rhodamine 6G gave a positive response in the absence of S9 in the mouse lymphoma assay for induction of trifluorothymidine (Tft) resistance in L5178Y cells; in the presence of S9, rhodamine 6G was negative. Rhodamine 6G induced sister chromatid exchanges (SCEs) and chromosomal aberrations in cultured CHO cells in the presence, but not the absence, of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* for male F344/N rats administered rhodamine 6G, as indicated by a marginally increased incidence of integumentary keratoacanthomas. There was *equivocal evidence of carcinogenic activity* for female F344/N rats administered rhodamine 6G, as indicated by a marginal increase in pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice administered 500 or 1,000 ppm rhodamine 6G in the diet.

There were no significant nonneoplastic lesions attributed to rhodamine 6G administration to male or female rats or male or female mice. Male and female rats might have been able to tolerate a higher concentration of rhodamine 6G in the feed.

Synonym: 2-[6-(ethylamino)-3-(ethylimino)2,7-dimethyl-3*H*-xanthen-9-yl] benzoic acid ethyl ester, monohydrochloride

Common Names: Basic Red 1; Basic Rhodamine Yellow; Basic Rhodaminic Yellow; Calcozine Red 6G; Calcozine

Rhodamine 6GX; C.I. Basic Red 1, Monohydrochloride; Elcozine Rhodamine 6GDN; Eljon Pink Toner; Fanal Pink GFK; Fanal Red 25532; Flexo Red 482; Heliostable Brilliant Pink B extra; Mitsui Rhodamine 6GCP; Nyco Liquid Red GF; Rhodamine 69DN Extra; Rhodamine F4G; Rhodamine F5G; Rhodamine F5G chloride; Rhodamine 6GB; Rhodamine 6GBN; Rhodamine 6GCP; Rhodamine 6GD; Rhodamine 4GD; Rhodamine GDN; Rhodamine 5GDN; Rhodamine 6 GDN; Rhodamine GDN Extra; Rhodamine 6GEx ethyl ester; Rhodamine 6G Extra; Rhodamine 6G Extra Base; Rhodamine 4GH; Rhodamine 6GH; Rhodamine 5GL; Rhodamine 6G lake; Rhodamine 6GX; Rhodamine J; Rhodamine 6JH; Rhodamine 7JH; Rhodamine Lake Red 6G; Rhodamine Y 20-7425; Rhodamine Zh; Rhodamine 6ZH-DN; Silosuper Pink B; Valley Fast Red 1308

Report Date: September 1989

TR-365 Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate (CAS No. 78-11-5) with 80% D-Lactose Monohydrate (PETN, NF) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Pentaerythritol tetranitrate (PETN, NF) is a drug used to prevent angina pectoris. PETN without a lactose stabilizer is used as an explosive. Toxicology and carcinogenesis studies were conducted by administering PETN, NF, to groups of F344/N rats and B6C3F$_1$ mice of each sex once by gavage or in feed for 14 days, 13 or 14 weeks, or 2 years. The PETN component was greater than 99% pure. Genetic toxicology studies were conducted with *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 14-day studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats were comparable. The final mean body weight of female mice that received 50,000 ppm was 13% lower than that of controls. No clinical signs or toxic lesions were attributed to PETN, NF, administration.

All rats and mice lived to the end of the 13-week (mice) and 14-week (rats) studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats and mice were similar, although weight gains of female rats at 25,000 and 50,000 ppm were less than that of controls. The nitrite level in urine of rats and methemoglobin levels in whole blood of rats and mice were not affected by administration of PETN, NF. An adenoma of the Zymbal gland was seen in a female rat that received 50,000 ppm. A hepatocellular adenoma was seen in a female mouse that received 50,000 ppm.

Based on these results and the NTP convention of limiting concentrations in 2-year feed studies to 5% of the diet, the 2-year studies were conducted by administering 0, 25,000 or 50,000 ppm PETN, NF, in feed for 104 weeks to groups of 50 male rats and for 103 weeks to groups of 49 or 50 mice of each sex. Groups of 50 female rats were given feed containing 0, 6,200, or 12,500 ppm PETN, NF, for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 2%-9% lower than those of controls throughout the study; body weights of all groups of female rats were similar. No significant differences in survival were observed between any groups of rats of either sex (male: control, 23/50; low dose, 29/50; high dose, 29/50; female: 33/50; 33/50; 31/50). Mean body weights of dosed and control mice were similar. The survival of both groups of dosed male mice was significantly greater than that of the controls (26/49; 38/50; 38/50). No significant differences in survival were observed between any groups of female mice (38/50; 30/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No nonneoplastic lesions were attributed to PETN, NF, administration in rats or mice. Neoplasms of the Zymbal gland occurred in dosed male (control, 0/49; low dose, 3/45; high dose, 2/41) and dosed female (0/36; 1/37; 3/35) rats. The historical incidence of these neoplasms is 1% ± 2% in untreated males and 0.6% ± 1% in females.

At no site was a significantly increased incidence of neoplasms observed in dosed male or female mice.

Genetic Toxicology: PETN, NF, was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation (S9). When tested for cytogenetic effects in cultured CHO cells, PETN, NF, induced sister chromatid exchanges (SCEs) in the presence and absence of metabolic activation; no induction of chromosomal aberrations was observed in CHO cells with or without activation.

Audit: The data, documents, and pathology materials from the 2-year studies of PETN, NF, have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of PETN, NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland. Female rats might have tolerated a higher dose. There was *no evidence of carcinogenic activity* of PETN, NF, for male or female B6C3F$_1$ mice fed diets containing 25,000, or 50,000 ppm for 2 years. No nonneoplastic lesions were attributed to PETN, NF, administration.

Synonyms for PETN: 2,2-bis((nitrooxy)methyl)-1,3-propanediol dinitrate (ester); 2,2-bisdihydroxymethyl-1,3-propanediol tetranitrate; niperyt; nitropentaerythritol; pentaerythrityl tetranitrate; penthrit

Trade Names for PETN, NF: Angitet; Cardiacap; Dilcoran-80; Dipentrate; Hasethrol; Lentrat; Metranil; Mycardol; Neo-Corovas; Nitropenta; Nitropenton; Pen-